THE PLANT DISEASE REPORTER

Issued By

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

LIBRARY
CURRENT SERIAL RECORD
SEP 17 1958
U. S. DEPARTMENT OF AGRICULTURE

Volume 42 **Number** 8

August 15, 1958

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

SUGGESTIONS FOR PREPARATION OF MANUSCRIPTS FOR THE PLANT DISEASE REPORTER

(1) GENERAL: The Reporter page measures 9 inches long with the heading or 8 3/4 inches for the text part, by 6 inches wide. The copy is typed on a larger page, 11 1/4 inches of text or 12 inches overall in length by 8 inches in width, and reduced 25 percent in the photographic process of reproduction. Illustrations or tables larger in either dimension will take a correspondingly greater reduction. Only one size of type is available for text, footnotes, or tables.

(2) MANUSCRIPTS should be the original ribbon copy, not carbons, clearly typed and double-spaced throughout, including tables, footnotes, and bibliographies. (Note -- only one copy is needed.) Footnotes should be typed at the bottom of the page.

(3) ABSTRACTS are requested for all except very short articles.

(4) CAUSES OF DISEASES should be named. For bacteria, fungi, nematodes, etc., give the Latin name of the organism; for viruses either or both the accepted common name of the virus or a Latin name if you prefer it and there is one; for non-parasitic diseases state the causal factor if it is known. If the cause of a disease has not been determined say so.

(5) LITERATURE REFERENCES should be given in alphabetical order and numbered for citation in the text. We follow the AIBS suggestion of placing the year of publication after the author's name. Please check your references carefully since we cannot do it for you. Be sure that text citations and bibliography agree; that foreign-language references are correct; that number or month is cited for periodicals that are not paged consecutively throughout the volume.

(6) NAMES OF FUNGICIDES should be given according to the suggestion of McCallan et al. in Phytopathology (45 (6): 295-302. 1955).

(7) ILLUSTRATIONS should be sent to us unmounted. To prevent mistakes, write figure numbers on the back, and mark the top of each print when necessary. A sketch can show a preferred arrangement but please keep in mind page size, shape, and standard reduction (see above under General), and remember that figure titles and legends are part of the page. Lettering should be clear and large enough to be legible after reducing. Drawings, maps and graphs can be photographs or originals, but should be finished and ready for reproduction, not just sketches.

(8) TABLES should be carefully thought out with particular attention to the Reporter's limitations in reproduction. Make titles and headings definite and self-explanatory. Designate footnotes in tables with superscript lower-case letters. Be sure that text discussion agrees with the data in the table. Do not abbreviate names of crop varieties.

(9) REPRINTS cannot be supplied since there is no way in which we can be reimbursed. However,

(10) The MULTILITH PLATES from which reprints can be made will be sent if requested at the time the article is submitted. The press size of these plates used for the Reporter is designated as small -- maximum image 9 1/2 by 13 inches, maximum paper size 9 3/4 by 14 inches -- for Model 1250. Most of the Experiment Stations have this type of multilith machine.

ACCEPTANCE OF MANUSCRIPTS

The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to 15 double-spaced typed pages. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases. By following this procedure we hope to continue publishing all articles promptly.

Paul R. Miller

Manuscripts for and correspondence about this publication should be sent to:

PLANT DISEASE REPORTER
Mycology and Plant Disease Reporting Section
Crops Protection Research Branch
Plant Industry Station
Beltsville, Maryland

THE PLANT DISEASE REPORTER

Crops Research Division — Plant Industry Station, Beltsville, Maryland
Volume 42 — August 15, 1958 — Number 8

IN THIS ISSUE

VIRUS (see also Sinclair et al., page 984; Leyendecker, page 989):

Studies comparing New York and Washington colonies of the English grain and apple-grain aphids for efficiency in transmitting the barley yellow dwarf virus in both States indicate that the prevalence of differential transmission in New York and its relative scarcity in Washington are associated with regional differences in the virus complex. W. F. ROCHOW reports the New York results, page 905; G. W. BRUEHL reports the work in Washington, page 909.

W. H. SILL Jr. summarizes symptoms, transmissibility, and other characteristics of soil-borne wheat mosaic viruses, page 912.

Reaction of various plants to inoculation indicates that the naturally occurring symptomless virus of the Kwanzan and Shiro-fugen flowering cherries is different from the western X-disease virus, according to E. L. REEVES and PHILIP W. CHENEY, page 925.

D. C. GIACOMETTI and A. S. COSTA report studies with mild and severe strains of the citrus tristeza virus in Brazil, page 928.

Tests by C. W. ANDERSON showed that a ringspot virus obtained from hydrangea plants in Florida was related to the tobacco ringspot virus, page 932.

NEMATODE:

W. A. FEDER et al. found no immunity and little tolerance among citrus forms and relatives screened for susceptibility to attack by the burrowing nematode, page 934.

Results of a survey to determine nematodes associated with banana root injury in Honduras are reported by R. H. STOVER and MAX J. FIELDING, page 938.

D. J. RASKI and J. D. RADEWALD state that of four different ectoparasitic nematodes from California vineyard soils three proved to be definitely parasitic on grape roots, and of these three only Xiphinema index caused obvious root injury, page 941.

HAROLD W. REYNOLDS lists rotation, summer fallow, and soil fumigation as effective control measures for cotton root-knot nematode on extra-long staple cotton in the Southwest, page 944.

CEREALS, FORAGE CROPS (see also under Virus, Unusual Records):

J. ARTIE BROWNING discusses some new sources of resistance to several races of the oat stem rust fungus, page 948.

In JOHN J. NATTI's trials of seed treatment with various antibiotics to control bacterial wilt of corn no material gave effective control without causing injury to the seedlings, page 953.

By extraction from wheat and barley seeds, PETER A. ARK and J. P. THOMPSON obtained a substance that inhibited growth of Gram-positive bacteria and of some fungi, page 959.

Susceptibility of Ladino white clover to damping-off was increased by seed treatment with gibberellic acid in experiments reported by J. H. GRAHAM, page 963.

VEGETABLE CROPS (see also Unusual Records):

HARRY C. FINK reports that potato seed-piece treatment with streptomycin-fungicide mixtures did not control blackleg rot in field tests although it had given promising results in laboratory trials, page 965.

RICARDO A. RODRÍGUEZ describes the "torbó" disease of potato which is serious in Costa Rica; inoculation experiments indicate that the cause is a species of Rosellinia, page 972.

Occurrence of Botrytis blight of pepper (Capsicum) in North Carolina is reported by N. N. WINSTEAD, J. C. WELLS, and W. W. REID, page 981.

An undetermined distal-end rot affecting the Acadian sweetpotato variety is described by W. J. MARTIN, page 983.

Aster yellows of shallot and certain diseases of pepper and tomato were unusually prevalent in Louisiana during the 1957-58 growing season, according to J. B. SINCLAIR, N. L. HORN, and E. C. TIMS, page 984.

ORNAMENTALS, TREES (see also under Virus):

PHILIP BRIERLEY and PAUL LORENTZ describe a dieback of gardenia, attributed to overwatering, page 986.

T. W. JONES and T. W. BRETZ report dying of shortleaf pine in Missouri, caused by Fomes annosus root rot, page 988.

MISCELLANEOUS:

Unusual Records, page 989: Big vein of lettuce discovered in New Mexico, by PHILIP J. LEYENDECKER, Jr. Reaction of rhubarb and buckwheat to inoculation with reed grass rust, by JOHN W. BAXTER.

Book Review: "Diseases of Tobacco", by George Blanchard Lucas, reviewed by HILDE McGRATH, page 990.

Announcement: New location of Chicago Market Pathology Laboratory, page 991.

June Weather, page 992.

THE ROLE OF APHIDS IN VECTOR SPECIFICITY OF BARLEY YELLOW DWARF VIRUS[1]

W. F. Rochow[2]

Summary

Colonies of aphids from New York and from Washington were compared as vectors of five "vector specific" isolates of barley yellow dwarf virus. Tests involved acquisition feeding on detached half-leaves or the rearing of aphids on virus source plants. Virus transmission by apple grain aphids regularly used in New York and by apple grain aphids from Washington was identical in all but 1 of 16 comparisons. Virus transmission by English grain aphids from the two areas was identical in all but 3 of 20 tests. These data are considered as evidence that the basis for the observed vector specificity of barley yellow dwarf virus rests with the virus and not with the vector.

Vector specific isolates of barley yellow dwarf virus have been reported from Washington (3) and from New York (2). Virus was recovered by both apple grain and English grain aphids from most of the field samples collected by Toko and Bruehl in Washington, but one isolate was transmitted only by apple grain aphids and another only by English grain aphids (3). In New York, however, virus was transmitted from field samples usually only by English grain aphids (2).

To determine whether variability between the aphids used in New York and in Washington was a major factor in this specificity, tests were made in cooperation with G. W. Bruehl, whose report on similar studies appears separately (1). Apple grain and English grain aphids collected in Washington were sent to the writer by Dr. Bruehl. Colonies of the same aphid species regularly used in New York and the colonies from Washington were compared by means of transmission tests with isolates of barley yellow dwarf virus currently under study in this laboratory. Results of these comparisons are the subject of this paper.

MATERIALS AND METHODS

Stock colonies of virus-free apple grain (AG) aphids (Rhopalosiphum fitchii (Sand)) and English grain (EG) aphids (Macrosiphum granarium (Kirby)) were maintained on caged plants in a laboratory to isolate them from plants infected by barley yellow dwarf virus (BYDV). New colonies of each collection were started several times each week. Viviparous females were placed on an oat or barley seedling in a closed plastic dish containing moist filter paper, and newly emerged nymphs were transferred to seedlings of Dubois oats or of Erie barley in the one- to two-leaf stage. Seedlings were grown in 4- or 6-inch pots. After the plants were infested with 10 to 15 nymphs, they were covered with pot cages made from Pyralin cellulose nitrate plastic and Lumite saran screen. Pots were placed either on a window sill or under continuous fluorescent illumination. When sufficient aphids had developed in a colony (usually after about 4 weeks), the cage was opened for the first time, the aphids were used, and the plants were discarded. Colonies maintained in this way have remained nonviruliferous for over a year, but separate aphid controls always were included for each colony used.

Three colonies of AG aphids, each of which had a different origin, were used in these studies. The first, which originally was supplied by R. M. Endo, is the colony regularly used in all New York studies. Unless stated otherwise, it is the New York colony used in the tests described here. The second AG colony originated from a collection made by the writer near

---

[1] Cooperative investigations between Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Cornell University Agricultural Experiment Station.

[2] Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture. Grateful acknowledgment is made to David Zipster and Richard Sievert for part-time assistance.

Ithaca, New York, in the summer of 1957. Previous comparison of these two colonies has shown that they act similarly in the transmission of virus from New York field samples (2). The third AG colony was supplied by G. W. Bruehl at the start of these investigations in November, 1957, and represents a collection of AG aphids from Washington.

Two colonies of EG aphids were used. The first was collected by the writer in the field near Ithaca, New York, in the fall of 1956 and is the EG colony regularly used in work in this laboratory. The second EG colony was supplied by G. W. Bruehl from Washington in November, 1957.

Five different isolates of BYDV were used in the comparisons of these aphids. All of these virus isolates were obtained from New York oats in the summer of 1957 in tests reported previously (2). Each isolate originally was transmitted from a single leaf collected in the field and tested with both AG and EG aphids by the detached-half-leaf method (2). Three of the isolates were transmitted from the field samples only by EG aphids. One of these isolates (EG-1) was obtained from Broome County, a second (EG-2) was isolated from a leaf collected at Caldwell Field on the University Farm, and the third (EG-3) originated from the Cornell Tailby Farm. Two of the isolates were transmitted originally only by AG aphids. The first of these isolated (AG-1) was from Broome County, and the second (AG-2) was from Caldwell Field on the University Farm.

In separate studies on the nature of this vector specificity, each of the above virus isolates generally has retained its original vector specificity in repeated transfers. The general failure of AG aphids to transmit "EG-specific" isolates has been particularly striking. In all cases, however, transmission of virus from an isolate by the "nonvector" aphid has occurred at least a few times. The transmission of "AG-specific" isolates by EG aphids has occurred fairly often and can be noted in the results reported below. In other words, as was indicated originally (2), the "AG-specific" isolates appear to be less vector specific than are the "EG-specific" isolates.

Most of the experiments described in this paper were based on use of the detached-half-leaf technique (2), except that plastic dishes with tight-fitting covers were used in some tests instead of Petri dishes. In a typical experiment, two adjacent leaves were removed from an oat plant infected with one of the isolates of BYDV. One leaf was cut in half longitudinally, and opposite half-leaves were placed in separate plastic dishes. One half-leaf was infested with New York AG aphids; the opposite half-leaf was infested with Washington AG aphids. The second leaf was treated in the same way except that the two colonies of EG aphids were used instead of AG aphids. In each experiment, a comparable group of aphids was placed on healthy seedlings of oats or barley to serve as nonviruliferous controls. The dishes were stored in the laboratory, usually in the dark, for an acquisition feeding period of at least 24 hours. Aphids were then transferred to test seedlings (California Red oats, C. I. 1026) in groups of about 10 aphids per seedling for a 3-day test feeding. Most of the aphids in each group usually were mature apterous females, but other forms were also included. At the end of the test feeding period, the plants were fumigated with lindane in a closed chamber and placed on a greenhouse bench under supplemental illumination for at least 4 weeks. Observations were made weekly during this period.

The Eastern and Western aphids also were compared in an experiment in which acquisition feeding was by rearing aphids on source plants. Two comparable California Red oat plants infected with one of the virus isolates were selected. One plant was infested with a mixture of AG and EG aphids from the colonies regularly used in New York, and the other plant was infested with a mixture of the AG and EG aphids from Washington. Aphids were removed from each plant after 10 days and the AG and EG aphids were separated by means of a camel's-hair brush. Aphids were transferred to test seedlings in groups of five aphids per seedling for a 3-day test feeding. Observations were made weekly for at least 4 weeks after removal of the aphids.

RESULTS

Transmission of the five isolates of BYDV from opposite half-leaves was compared 16 times in six experiments carried out over a period of 5 months. In each experiment controls showed that all aphids were nonviruliferous at the start of the test. Results of 16 comparisons of two AG colonies were identical (Table 1). Results also were identical in 14 of 16 comparisons of EG aphids from the New York and Washington collections (Table 1). In the two exceptions, partial transmission of an "AG-specific" isolate occurred with Washington EG aphids in one case and with New York EG aphids in the other. These results show that there are no major differences between the Eastern and Western collections of each aphid species with respect to

Table 1. Transmission of five isolates of barley yellow dwarf virus from detached half-leaves by colonies of New York and of Washington aphids.

New York virus isolate	Number of test plants infected following tests with aphid indicated[a]				
	AG			EG	
	New York[b]				
	A	B	Washington	New York	Washington
EG - 1		0/3	0/3	3/3	3/3
EG - 1	0/3	0/3		3/3	3/3
EG - 2		0/3	0/3	3/3	2/2
EG - 2		0/3	0/3	3/3	3/3
EG - 2		0/3	0/3	3/3	3/3
EG - 2	0/3	0/3		3/3	3/3
EG - 3		0/3	0/3	2/2	2/2
AG - 1		4/4	4/4	0/3	0/3
AG - 1		3/3	3/3	0/3	0/3
AG - 1		3/3	3/3	0/3	0/3
AG - 1	3/3	3/3		0/3	0/3
AG - 2		3/3	3/3	0/3	0/3
AG - 2		3/3	3/3	1/3	1/3
AG - 2		4/4	4/4	1/4	1/4
AG - 2		3/3	3/3	0/3	2/3
AG - 2	3/3	3/3		1/3	0/3

[a]Numerator is number of California Red oat test seedlings that became infected; denominator is number of seedlings that were infested with about 10 apple grain (AG) or English grain (EG) aphids.

[b]New York AG aphids of A were collected in the field near Ithaca, New York. Those of B originally were supplied by R. M. Endo and represent the colony used regularly in work with the virus in this laboratory.

Table 2. Transmission of four isolates of barley yellow dwarf virus by colonies of New York and Washington aphids after aphids were reared on source plants.

New York virus isolate	Number of test plants infected following tests with aphid indicated[a]			
	AG		EG	
	New York	Washington	New York	Washington
EG - 1	0/6	0/6	5/5	6/6
EG - 2	0/6	1/6	6/6	6/6
AG - 1	3/3	3/3	0/6	0/6
AG - 2	3/3	3/3	0/6	2/6

[a] Numerator is number of California Red oat test seedlings that became infected; denominator is number of seedlings that were infested with 5 apple grain (AG) or English grain (EG) aphids.

transmission of the isolates of BYDV tested.

Results of the comparisons in which aphids were reared on source plants (Table 2) also indicated that the Eastern and Western collections of each aphid species do not differ in their ability to transmit the isolates of BYDV tested. Results were identical in six of the eight comparisons. The two exceptions are considered as examples of the occasional transmission of an isolate by the "non-vector" aphid. Although both of these exceptions involved Washington aphids, the same sort of occasional transmission has been encountered with New York aphids in other tests. Controls showed that all aphids were nonviruliferous when they were placed on the source plants at the start of these tests.

DISCUSSION

The sum of several reports makes it clear that any differences that might exist among collections of AG or EG aphids in the transmission of BYVD are not sufficient to be an important factor in the vector specificity encountered so far with this virus. All present information shows that the basis for vector specificity rests with the virus and not with the vector.

In the first place, three different colonies of AG aphids originally had been found to act similarly in the transmission of BYDV from oats in New York (2). Secondly, Eastern and Western aphids gave identical transmission results in 32 of a total of 36 direct comparisons involving two different methods reported in this paper. Even in the four cases where results were not identical, no major differences between colonies were indicated. Thirdly, Eastern and Western aphids, AG as well as EG aphids, were equally effective in transmission of BYDV from 28 samples tested by Bruehl in Washington (1).

The data reported in this paper illustrate further the extent of vector specificity of some isolates of BYDV. In the tests with the "EG-specific" isolates, for instance, 62 of 62 test plants were infected when EG aphids were used as vectors but only 1 of 66 plants was infected when AG aphids were used as vectors in comparable tests.

Literature Cited

1. BRUEHL, G. W. 1958. Comparison of Eastern and Western aphids in the transmission of barley yellow dwarf virus. Plant Disease Reptr. 42: 909-911.
2. ROCHOW, W. F. 1958. Barley yellow dwarf virus disease of oats in New York. Plant Disease Reptr. 42: 36-41.
3. TOKO, H. V., and G. W. BRUEHL. 1957. Strains of the cereal yellow-dwarf virus differentiated by means of the apple-grain and the English grain aphids. (Abstr.) Phytopathology 47: 536.

DEPARTMENT OF PLANT PATHOLOGY, CORNELL UNIVERSITY, ITHACA, NEW YORK

COMPARISON OF EASTERN AND WESTERN APHIDS IN THE TRANSMISSION OF BARLEY YELLOW DWARF VIRUS[1]

G. W. Bruehl[2]

Summary

Apple grain and English grain aphids collected in New York and Washington were compared as vectors of 28 collections of the Washington barley yellow dwarf virus. Aphids of each colony transmitted each of the 28 collections of virus. These results show that the Washington collections, for the most part, have little or no vector specificity in regard to these two aphid species. Aphids from New York were equal as vectors to those of Washington, being in no way distinguished in these tests. These observations with those of Rochow (4) indicate that the prevalence of "vector-specific" strains in New York and their relative absence in Washington is due to a regional difference in the virus complex.

The predominance of strains of BYDV adapted for transmission by the English grain aphid in New York and the relative absence of "vector-specific" strains in Washington may reflect a difference in aphid species dynamics. The hypothesis is advanced that the English grain aphid may have predominated for years in New York, but aphid species may have fluctuated abruptly and markedly in Washington. This latter situation would place selection pressure on the virus toward versatility in vector relationships.

Oswald and Houston (2) proved that several aphids are good vectors of the barley yellow dwarf virus (BYDV) in California. Marion Watson and co-workers reported that all species found breeding on cereals and grasses in England are vectors (8). Toko and Bruehl (6) observed a partial superiority in transmission of a single Washington strain of the BYDV by the apple grain aphid (Rhopalosiphum fitchii Sand.) when compared with the English grain aphid (Macrosiphum granarium Kirby). This partial difference in vection stimulated a search for strains of the virus which might be vector-specific, i. e. transmitted by only one or the other species of aphids, but not by both. Two such strains were found in a study of 34 Washington collections (7), one AG (apple grain transmitted) and one EG (English grain transmitted) strain. Both aphids were effective vectors of the remaining 32 BYDV collections. Vector-specificity was viewed as a rare occurrence. The EG and AG strains were thought to represent a rare circumstance in nature, but nothing of practical significance.

Rochow (3) very shortly thereafter reported that a rather high vector-specificity prevailed among BYDV collections in New York State. Of 29 accessions of diseased oats in New York, he found that the virus of 26 of them was effectively transmitted by the EG aphid only, that 2 were more or less specific to the AG aphid, and that both species of aphids were effective vectors of only 1 of the 29 collections studied.

The contrasting data now at hand, i. e. vector-specificity rare in Washington but the rule in New York, were impossible to interpret. Were these differences due to variations in the abilities of different clones of the aphid species to act as vectors, or were the differences due to a regional variation in the virus complex itself? Exchanges of non-viruliferous aphids between New York and Washington were employed to resolve this question.

---

[1]Scientific paper No. 1740, Washington Agricultural Experiment Stations, Pullman; work conducted under Project No. 1280. The cooperation of the Agricultural Research Service, Section of Forage Crops, is gratefully acknowledged.

[2]Associate Pathologist, Washington Agricultural Experiment Stations.

MATERIALS AND METHODS

In the fall of 1957 English grain and apple grain aphids were collected near Pullman, Washington and increased on oats in the greenhouse. Adults were then transferred to Petri dishes containing moistened filter paper (2). The virus-free nymphs borne in this manner were transferred to healthy seedling Shasta oats (Avena sativa L., C. I. 3976) and increased thereafter on fresh oats as needed. Plastic mesh screened cages were used to confine the colonies. The cage bottoms were ringed with cotton as described by Allen (1) to better confine the aphids. The pots were watered in saucers, removing the necessity of opening the cages for this purpose. Virus-free apple grain and English grain aphids of New York were obtained from W. F. Rochow (4) and increased in a similar manner.

Yellow dwarf plants were collected during late summer and fall. The inflorescences were removed to retain vegetative vigor as long as possible. The plants were potted, brought to the greenhouse, and fumigated regularly to prevent mix-up of the virus materials. Some of the severely diseased plants, especially those nearly mature when collected, died before testing, but 28 remained vigorous and constituted a fair random sample of the BYDV as it occurs in Washington. The virus sample consisted of 21 cultivated oats, 2 barley, 4 wild oats, and 1 volunteer winter wheat plant. Sixteen oat (Avena sativa L.) plants were from Whitman County, 2 from Benton County (near Prosser), 2 from Clark County (near Vancouver), and 1 from Lewis County (near Ethel). Two barley plants (Hordeum vulgare L.) were from Clark County (near Vancouver); 2 wild oats (A. fatua L.) from Lewis County (near Toledo), 1 from Skamania County (near Stevenson), and 1 from Columbia County (near Long). The 1 volunteer winter wheat plant (Triticum aestivum L.) was from Klickitat County (near Goldendale).

The split-leaf acquisition technique of Rochow (3) was slightly modified. Glass-stoppered vials about 2 1/2 inches in height and with about 1/2-inch inside diameter were used to confine the test aphids on pieces of leaf from a given source plant. A small wad of cotton was forced down to the bottom of the vial, tamped to lessen the cotton wisps which entangle and exhaust caught aphids, and 1/2 ml of water was pipetted in. Usually, two to three leaf pieces about 2 to 3 cm in length were placed in a vial and at least 10 aphids introduced by means of a camel's hair brush. The vials were then held at room temperature in subdued light or darkness for 2 days. After this acquisition feeding the leaf pieces were carefully removed, working over glossy white paper. All vigorous aphids which were feeding on the leaf pieces were transferred to healthy seedling Shasta oats. The test seedlings were caged in the greenhouse for a 48-hour transmission period, fumigated, then removed to another greenhouse section for growth. This section was periodically fumigated.

Toko (unpublished Thesis, Library, Washington State College) had obtained good virus acquisition from various portions of the shoot of diseased oats with both the AG and EG strains (7), so the author did not split the leaf segments longitudinally as Rochow (3) had done. Leaf segments were cut to fit the vial.

The aphid colonies were periodically checked to assure that they remained virus-free. Rochow's detached leaf technique has the real advantage that, should a colony become viruliferous, the test plants would not be contaminated with added introduced virus. Contamination in this manner is a definite possibility because tests to date (1, 7) give no evidence of interference between BYDV strains. All trials giving positive transmission were not repeated.

In working with aphids enclosed in glass vials avoid excess water. The aphids tend to become trapped in condensation droplets, and exhaust themselves fighting surface tension. The vials were autoclaved between usages as a precaution against building up harmful microorganisms in the moist interior.

RESULTS

As all vigorous feeding aphids were transferred to one or more seedlings in a single pot, no data on relative efficiency were obtained; only the ability to transmit the test virus was recorded. The virus of all 28 plants of the random sample of Washington BYDV was transmitted by aphids of each of the four aphid colonies.

DISCUSSION

The earlier results (7) indicating that the major portion of the BYDV as constituted in Washington is transmitted by either apple grain or English grain aphids were confirmed. The ability of the New York clones of these two aphids to effectively transmit these same collections rules out the possibility that the different results obtained by Rochow (3) in New York are explainable on the basis of differences in transmission abilities of the aphids used. The use of both Washington and New York aphids by Rochow (4) in a recheck of some relatively vector-specific New York virus collections and their identical performance as vectors likewise confirms the similar abilities of these aphids of different geographic origin. This was further confirmed (4) by the tests with Endo's Illinois colony of apple grain aphids.

Elimination of the vector as a possible explanation of the abundance of vector-specific strains in New York (3) and the paucity of vector-specific strains in Washington (7) points to the existence of geographic variations within the virus complex itself. The lack of vector-specificity in the Washington virus represents greater virus adaptability, and should be a mechanism increasing its chances of survival. Possibly such differences reflect highly fluctuating populations of aphid species in Washington. Aphid population maxima may shift rapidly from species to species, applying selection pressure to the virus population, favoring and increasing those virus entities capable of transmission by two or more aphid species. In New York the aphid population may have been more stable through the years, dominated by the English grain aphid. If so, little selection pressure in the direction of vector-adaptibility would have been applied to the virus.

The predominance of "vector-specific" strains of the BYDV in New York (3, 4) complicates the problem of definite diagnosis. Suspected cases of BYD could not be definitely disproven unless subjected to test-feeding by more than one species of vector. Thus, it would have been misleading for Rochow to rely on the apple grain aphid alone in testing collections of BYD plants in New York. On the other hand, in Washington such a procedure apparently would be quite safe. In initiating studies in new areas caution in this respect is necessary.

The extensive California studies (1, 2) and those in Illinois (5) where no real difficulties of transmission were encountered, indicate that the virus in these areas is more or less similar to that of Washington, able to "ride" with whatever grass aphid comes along.

Literature Cited

1. ALLEN, THOMAS C., Jr. 1957. Strains of the barley yellow-dwarf virus. Phytopathology 47: 481-490.
2. OSWALD, J. W., and B. R. HOUSTON. 1953. The yellow-dwarf virus disease of cereal crops. Phytopathology 43: 128-136.
3. ROCHOW, W F. 1958. Barley yellow dwarf virus disease of oats in New York. Plant Disease Reptr. 42: 36-41.
4. ROCHOW, W. F. 1958. The role of aphids in vector specificity of barley yellow dwarf virus. Plant Disease Reptr. 42: 905-908.
5. TAKESHITA, R. M. 1956. Identity of the oat red-leaf virus with the barley yellow-dwarf virus. Phytopathology 46: 436-440.
6. TOKO, H. V., and G. W. BRUEHL. 1956. Apple-grain and English grain aphids as vectors of the Washington strain of the cereal yellow-dwarf virus. Plant Disease Reptr. 40: 284-288
7. TOKO, H. V., and G. W. BRUEHL. 1957. Strains of the cereal yellow-dwarf virus differentiated by means of the apple-grain and the English grain aphids. (Abstr.) Phytopathology 47: 536.
8. WATSON, MARION, J. T. SLYKHUIS, and T. E. MULLIGAN. 1956. Virus diseases of cereals in Great Britain. Commonwealth Phytopathology News 4: 6-7.

WASHINGTON AGRICULTURAL EXPERIMENT STATION, PULLMAN, WASHINGTON

A COMPARISON OF SOME CHARACTERISTICS OF SOIL-BORNE WHEAT MOSAIC VIRUSES IN THE GREAT PLAINS AND ELSEWHERE[1]

W. H. Sill, Jr.[2]

Summary

Evidence presented would indicate that the various soil-borne wheat mosaic viruses reported in the United States are probably strains of the same virus. No satisfactory evidence for more than one strain in infested Kansas soils has been found as yet, although two have been reported in Illinois and eastward. The rosette reaction characteristic of a few susceptible wheat varieties elsewhere has not been seen thus far in Kansas. In other respects the characteristics compared are quite similar. Based upon the evidence available the soil-borne wheat virus of Japan would seem to be closely related also. The soil-borne oat mosaic virus of the southeastern United States appears to be similar except for host range and some symptoms; however, it probably should be considered a distinct virus until more complete data are available.

Soil-borne wheat mosaic viruses have been studied by many workers since first observed in 1919 (10, 16) in Illinois and Indiana. The virus nature of the rosette disease of wheat was recognized by 1925 (17, 25). Since that time apparently similar viruses have been reported in Virginia 1925, Maryland 1927, North Carolina 1929, Iowa 1950, Kansas 1949-51 (26), South Carolina 1944 (1), and Missouri 1944 (4). McKinney (20) also indicated that a soil-borne wheat virus probably was observed by Peltier at Lincoln, Nebraska, as early as 1922.

In Kansas between 1949 and 1951 several diseased wheat fields were observed (26). In 1952 a general epiphytotic occurred, both in Kansas (5) and in Oklahoma (40). The infested regions extended across State lines at various places, north into eastern Nebraska and south into Oklahoma. Field symptoms and disease development and severity were generally similar throughout the areas observed in all three States. A comparison of field and plant symptoms was made (5) and these were essentially as described by McKinney (20) and Koehler et al. (15) in Illinois and eastward, with the one exception that no rosette was observed in any wheat varieties. Since 1952 the author and others have made careful annual surveys in Kansas. Symptoms have remained essentially the same and no rosette has been seen.

Beginning in 1952 an attempt was made to compare the characteristics of the disease as it occurs in Kansas and the Great Plains with those reported from other areas of the United States and from Japan. This paper summarizes the research results in Kansas and makes pertinent comparisons with research done elsewhere when possible.

MATERIALS AND METHODS

Virus-infested soils were collected from Illinois, Nebraska, and many fields throughout eastern Kansas. Deep greenhouse flats were filled with these infested soils, planted to various varieties of susceptible wheats, and buried to the ground level. The plants were grown outdoors throughout the entire season from fall through spring. In other experiments plantings

---

[1]Contribution No. 519, serial No. 671, Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station, Manhattan.

The author extends his grateful thanks to Benjamin Koehler and W. M. Bever, of the Illinois Agricultural Experiment Station, who furnished infested soil samples from Illinois for this study; R. M. Caldwell, of the Indiana Agricultural Experiment Station at Purdue University, Lafayette, Indiana, who furnished seed of several wheat varieties; W. B. Allington, of the Nebraska Agricultural Experiment Station, who gave valuable advice and showed the author several virus-infested wheat fields in Nebraska south of Lincoln; and Hurley Fellows, Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, located at Kansas State College, who furnished constant advice, help, and inspiration.

[2]Associate Professor of Botany and Associate Plant Pathologist, Kansas State College, Manhattan.

were made in the greenhouse in 8-inch clay pots containing virus-infested soil. Some of these were kept in temperature control chambers held at near 60° F throughout the growing period as suggested by McKinney (20), while others were started outdoors and then moved into the temperature control chambers in January after vernalization (26). No attempt was made to control light or day length except that the temperature control chamber experiments were conducted only during the late fall, winter, and early spring to assure relatively short day lengths, which were reported by McKinney (20) as favorable for symptom expression.

Some other experiments were conducted in the field in virus-infested soils. In all field experiments only fall plantings were made and infection occurred naturally, presumably through the roots. Some infection by manual inoculation was achieved and will be discussed later, as well as some other methods which were used in specific experiments.

EXPERIMENTAL RESULTS AND COMPARISONS

Symptom Comparisons

In the field, symptoms have not been seen in the fall in Kansas but have developed very early in the spring with the start of active spring growth. This occurs in late February, March, or early April, depending upon the season. Usually symptoms have not appeared in the fall in other States, but Koehler et al. (15) and McKinney (20) have occasionally seen symptoms during long warm falls. When fields are first seen at a distance in the spring the areas of diseased wheat look light green or yellow green as compared to adjacent healthy wheat. A little later they may become yellow-bronze, a color which is distinctly different from typical early spring yellowing due to nitrogen deficiency. Shortly after hot weather commences the yellowing begins to disappear and, if hot weather persists, is soon gone. Then as the season progresses the diseased plant areas may be recognized by slight to moderate stunting, smaller heads, fewer heads, and retarded maturity when compared with healthy plant areas. Sometimes plants may make a remarkable recovery and show only very slight stunting and none of the other symptoms just mentioned. At harvest time the diseased areas are usually still green when all the nondiseased areas are ripe.

Diseased areas may vary in size from a few plants or an area a few feet across to those having a diameter of hundreds of feet. Occasionally whole fields of wheat may be infected. The diseased plant areas may be circular or irregular, on low or high ground, or on slopes. Often the infection seems to start and be initially more severe in the more moist portions of a field, poorly drained low spots, and waterways. It has also developed on only the well drained slopes and higher portions of other fields. Occasionally the disease has appeared to develop rapidly in a whole field in a single season (5). These observations concerning field symptoms and disease development are very similar to those reported by others (15, 16, 28) in Illinois and eastward.

Individual infected winter wheat plants develop symptoms rapidly in the spring under favorable environmental conditions. The predominantly yellow to bronze mosaic and mottle of early spring, characterized by tiny green islands of tissue in a lighter green to yellowish background and accompanied by some purpling of the leaf edges, rapidly changes with warmer weather to a greener mosaic, mottle, and some streaking. After considerable hot weather leaf symptoms may disappear entirely, but under more favorable conditions, they may persist even in the flag leaf as yellow-green or whitish streaks and blotches. The range of leaf symptoms as they appear on Pawnee wheat are shown in Figure 1; they are similar to the mosaic, mottling and streaking symptoms described by McKinney (20), Koehler et al. (15) and McKinney et al. (28).

Usually, diseased plants have not been killed in the early spring in Kansas, as reported in Illinois and eastward (15, 20). Only three fields among hundreds which have been seen during the 5-year observation period contained many plants apparently killed by the virus. No killing due to the virus was observed in 25 large experimental plots grown during 5 years. The fields with virus-killed plants appeared in the 2 years which were most favorable for virus development and later in the season became very weedy with much reduced stands. Similar weedy fields have been described in Illinois by Koehler et al. (15) and apparently occur frequently. Other symptoms in Kansas, such as delayed maturity, shorter heads, fewer heads, shriveled grain (Fig. 2), and moderate stunting, are similar to those described elsewhere.

The development of individual plant symptoms in Kansas has appeared to depend for the most part upon the susceptibility of the variety, the environment, and the level of virus infestation in a particular soil. This observation agrees essentially with earlier reports in other parts of the country by Moseman et al. (32), Koehler et al. (15), and McKinney (20). In

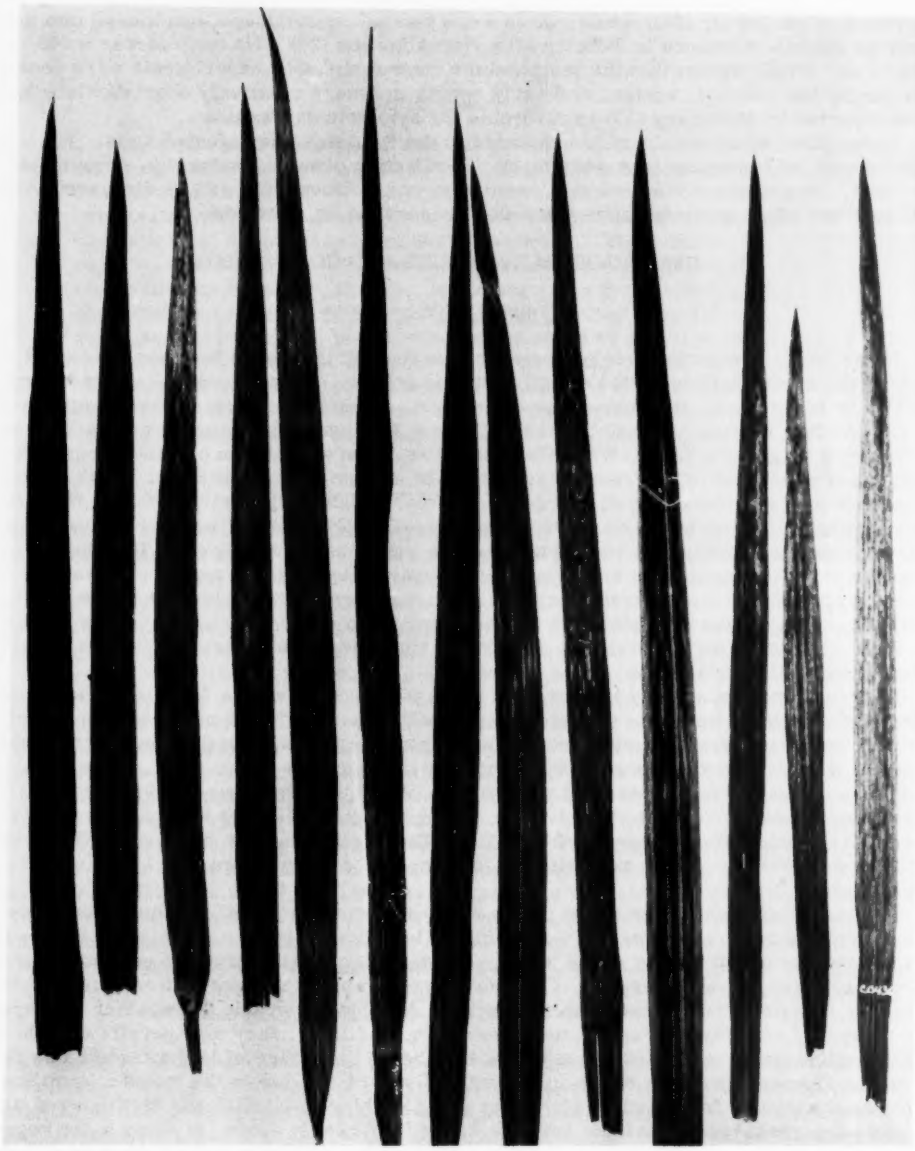


FIGURE 1. Range of soil-borne wheat mosaic virus leaf symptoms on Pawnee wheat grown in infested Kansas soils. Symptoms vary considerably and may be confused with those of other small grain virus diseases. Probably the best diagnostic symptom in Kansas is the dark green islands in a background of lighter green as shown on the fourth and fifth leaves from the left. The leaf at the extreme left is healthy.

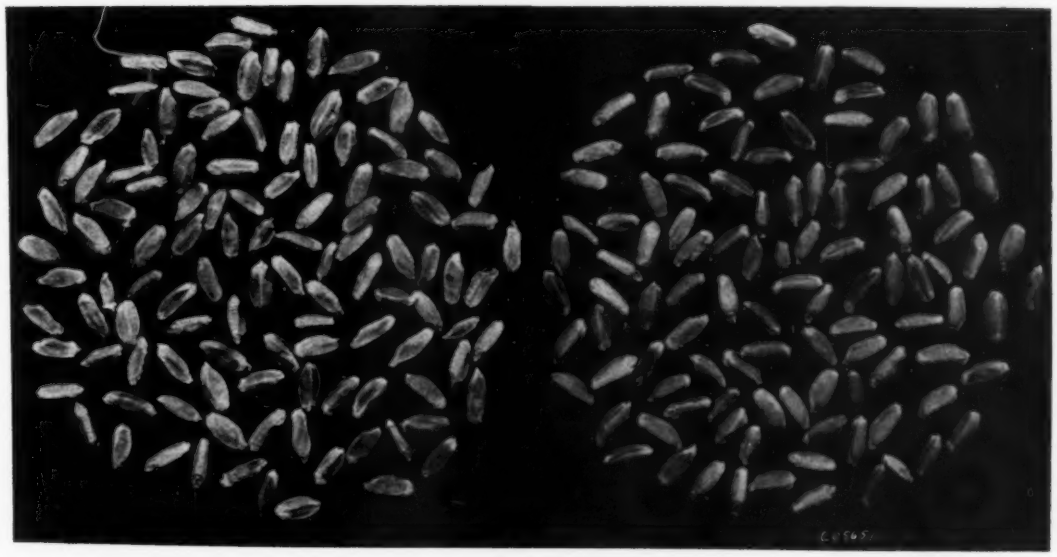


FIGURE 2. Shriveling in Ponca wheat due to infection with soil-borne wheat mosaic virus. Grain from diseased plants at left and from healthy plants at right. There was a yield reduction of approximately 25 percent in the diseased portion of the field from which the shriveled grain sample was taken.

certain wheat varieties both yellow and green mosaic, mottling, and streaking have been found in the field and seen in the various greenhouse and field plot experiments. However, based upon symptoms, no evidence for more than one strain of the virus has been found, although McKinney has reported two strains elsewhere (21). Symptom differences between various wheat varieties and in different environments have been great, but in similar or constant environments specific wheat varieties have given identical symptoms when grown in all virus-infested soils collected from the Great Plains and also in 25 experimental field plots. If more than one strain is present, it would appear then that they are distributed in all soils tested, the yellow and the green strains being intermixed.

One symptom reported in certain wheat varieties in Illinois and eastward (15, 20), which the author has never seen in the field in Kansas, is the rosette reaction. Rosetting in rosette-susceptible wheat varieties, such as Harvest Queen, has developed consistently for 3 years in environmental control chambers in Kansas when virus-infested soil from Illinois was used, but when these same varieties (including a very rosette-susceptible selection Purdue 45-1834) were grown under identical conditions in several virus-infested Kansas soils no rosette developed, only the typical green to yellow mosaic and mottle being present. If only the green strain described by McKinney (21) causes the rosette reaction, it would seem that it may be absent from the Great Plains.

Host Range Comparison

The extremely narrow host range of soil-borne wheat mosaic viruses in other regions has been characteristic of the virus in Kansas soils also. The following plants have been infected in both the field and the greenhouse during the past 5 years by growing them in various virus-infested soils: Several hundred varieties of winter wheat; two varietjes of winter rye (Balbo and an unknown variety found in Kansas which has given excellent and consistent symptoms (36)); and several varieties of spring wheat (Hope, Ceres, Marquis, Selkirk) which developed possible but very mild symptoms in the field but never in the greenhouse under controlled environment. Attempts to transmit the virus mechanically from these spring wheats to winter wheat in the greenhouse were always unsuccessful. Symptoms have not been seen in Kansas

in either the field or greenhouse on any of the barley varieties tested (Dicktoo, Reno, Flynn, Beecher, and Kearney), whether grown in Kansas or Illinois infested soils, nor have symptoms appeared in any of the oat varieties (Kanota, Fulton, New Nortex, Mo 0-205, Cherokee, and Clinton) tested during the 5 years. Some workers in the past have not reported symptoms on barley (1, 12, 34), whereas others have (15, 20). In Kansas symptoms have been observed on barley only once, in volunteer plants growing in a badly diseased wheat field, although hundreds of barley fields have been surveyed.

All workers listed above have reported oats to be immune and rye susceptible. Both Johnson (12) and McKinney (20) have noted occasional symptom development in spring wheats but only when planted in the fall. All attempts in Kansas to infect various native grasses with the virus have failed and no naturally diseased native grasses had been observed in infested fields until 1958, when an annual Bromus sp. showing symptoms was observed in a badly diseased wheat field. McKinney (20) reported one grass, Bromus commutatus Schrad., as susceptible.

Comparison of Virus-Infested Soils

Ten infested soils were compared for 5 years in experiments in the field, outdoors in pots and flats, and in the greenhouse under partially controlled environmental conditions. Seven of these were from Kansas, two from Illinois, and one from Nebraska. The Kansas soils were from widely separated infested fields in seven eastern counties. In each field where infested soil was collected plant symptoms were very severe throughout a large portion of the field. Hence, it was assumed that the virus infestation in each case was probably relatively uniform throughout. Each soil was a rather heavy upland or river bottom silt-loam. No virus-infested light or sandy soils have been found thus far in Kansas. This agrees with the observations of McKinney (17) and Koehler et al. (15).

The symptoms which developed on each of the wheat varieties of varying susceptibility grown in the ten soils were essentially identical during the 5 years and were similar to those described by others. Table 1 presents these results in detail. The characteristic varietal symptom differences under the controlled environmental conditions were far greater than the symptom differences owing to soil sources. In fact, it was impossible to note consistent symptom differences between soils when a given variety of wheat was grown simultaneously in the same environment in all ten soils. The only exception to this was that the rosetting varieties produced the rosette symptom when grown in Illinois soils but never in any of the others.

Some soils were typically more infectious than others, causing a higher percentage of plants to develop symptoms, and usually fresh soils were more infectious than soils from the same source stored in the greenhouse for 1 year or more. Several Kansas soils were still infectious after 4 years' storage in the greenhouse, but two had lost their infectivity by the second year and one became noninfectious the third year. McKinney (20) has reported dried soil infectious after 3 years, and soil in the field has been reported infectious up to 12 years (15, 25). In the field in Kansas particular soils have been infectious since 1949, although symptoms have been very mild during several years.

Comparison of Epidemiological Factors

During 6 years of careful observation soil-borne wheat mosaic virus epiphytotics in Kansas have developed in the eastern third of the State in the 1951-52 (5), 1953-54 (8), and 1956-57 (37) winter wheat seasons. These seasons were similar in several important respects, long cool growing periods during fall and spring, adequate to abundant moisture during the growing season, and open rather mild winters. By contrast, symptoms were very scarce and often absent during the 1952-53 and 1955-56 growing seasons (7, 35). Both years had a very dry fall and winter. Wheat emerged late in most areas. Both spring seasons were unseasonably warm and very dry, especially during the early spring. The 1954-55 season was intermediate as compared with the others observed (14). Wheat in eastern Kansas emerged fairly early in the fall and rains were adequate. Moisture during the winter and early spring was certainly not abundant but adequate for good wheat development. The early spring was cool and symptoms developed rapidly throughout the eastern counties. However, the symptoms disappeared quickly when hot and very dry weather began and persisted. Best late symptoms were seen along the Kansas River bottom in the low or naturally wetter spots in the infested fields. Even these symptoms gradually disappeared when the heat continued. These epidemiological observations in Kansas agree essentially with those of Webb (41), McKinney (20), and Koehler et al. (15).

Table 1. Symptoms on five wheat varieties of varying reaction grown under controlled environmental conditions in three virus-infested soils.[a]

Variety[b]	C.I. No.	Number of plants	Soil sources[c]			
			Illinois	Riley	Kansas mixture	Non-infested control
Royal	12558	32	--	--	g[d]	--
Atlas 66	12561	42	g	g	g	--
Pawnee	11669	61	yg	yg	yg	--
MoKing	12556	60	y	y	y	--
Harvest Queen (rosette-susc.)	several	34	gr	yg	yg	--
Purdue 45-1834		28	gr	yg	yg	--

[a]Growth chambers were held at an average temperature of 60° F. Light was not controlled except that all experiments were conducted during periods of short day length, from November to March. Optimum moisture conditions were maintained.

[b]Most resistant variety on top, becoming increasingly susceptible toward the bottom. This resistance pattern is similar to that reported by Koehler et al. (15) for most of the same varieties. The variety Atlas 66 was reported as susceptible by Fenne and Roane (6), but in the field and under the controlled conditions in the greenhouse it produced only green mosaic symptoms in Kansas and should be rated above Pawnee. The last variety, Purdue 45-1834, was furnished by R. M. Caldwell, of the Indiana Agricultural Experiment Station, who reported it via letter as the most rosette-susceptible variety he had seen.

[c]Illinois soil, a mixture of the two infested Illinois soils furnished by the Illinois Agricultural Experiment Station; Riley, an infested soil collected on the Agronomy Farm, Kansas Agricultural Experiment Station; Kansas mixture, a composite of all other infested Kansas soils discussed in the text.

[d]g = green mosaic symptoms only; y = yellow mosaic symptoms only; yg = both yellow and green mosaic present; gr = both green mosaic symptoms and rosetting present. Yellow mosaic symptoms predominated in the more susceptible varieties, except where rosette appeared. Green symptoms dominated in the more resistant varieties.

These authors reported long, cool growing periods, good moisture levels, and short days as producing optimum symptom development. Bretz (4) and Koehler et al. (15) also noted that the disease developed more frequently and consistently in low spots in infested fields and along river bottoms.

In Kansas the date of planting has not appeared to be of great importance to disease severity. Severe symptoms have been seen on wheat planted as early as September 15 and as late as November 10. During the 1955-56 season, the wheat planted in November was the only one of six plantings to develop symptoms. Plantings which have failed to develop symptoms during 5 years were planted both early and late, and in each case the unsatisfactory environmental factors previously discussed were present to account for the absence of symptoms. McKinney (16) and Johnson et al. (10) reported at least partial control by late seeding, and McKinney (20) suggested that either very early or very late sowing might reduce the severity of the soil-borne mosaics. In Kansas the critical environmental factors seem to be of much greater importance.

During a 5-year period repeated efforts were made with hundreds of plants to produce symptoms when susceptible wheat and rye varieties were grown in infested soils in the greenhouse at temperatures of 70° F or higher. No symptoms ever developed. All workers in other regions have reported similar results. An exception to this is the report of an unidentified rye variety which produced symptoms consistently in the greenhouse at temperatures of 70° F or slightly higher (36).

In four attempts with 109 Pawnee wheat plants no symptoms developed after manual abrasive inoculation when plants were grown in noninfested soil and kept in a chamber at low light intensity (1000 foot candles or less) and at 60° F. This was true also of 143 plants growing in

virus-infested soil under the same conditions. During one winter a prolonged cloudy period reduced light intensities so much that symptoms in all greenhouse test plants were diffuse or absent even though the average temperature was maintained at 60° F. It would appear that a mean light intensity at least above 1000 foot candles may be necessary for symptom development and certainly is necessary for good wheat growth. Satisfactory symptoms have developed in the greenhouse in all test plants only when temperatures were maintained at 60° F during the short but often bright days of late fall, winter, and early spring. These results are similar to those of McKinney (19) who reported symptoms developing best at 60° F with an 8-hour daily photoperiod.

Comparison of Methods of Transmission and Spread

All satisfactory, consistent infection in this work was obtained merely by planting susceptible wheat in virus-infested soil. As described previously the environment had to be satisfactory for symptom development. All workers with the soil-borne wheat mosaic viruses have reported similar results.

Abrasive Inoculation -- The six wheat varieties listed in Table 1 were grown at 60° F in virus-free soil during the winter. In three trials during three winters 82 plants were inoculated by the common carborundum leaf-rubbing method with freshly expressed sap from diseased plants. Only 12 of the 82 plants developed symptoms. Only one variety, Atlas 66, did not develop symptoms on any plant. None of the 62 uninoculated control plants developed symptoms. Both McKinney (17, 18) and Johnson (12) reported similar results, finding that abrasive inoculations produced a low percentage of infected plants. McKinney et al. (30) recently have reported more infectious virus from young diseased plants, both roots and tops, and higher percentages of diseased plants developing after manual inoculation.

Two attempts, using a total of 60 Pawnee wheat plants, were made to transmit the virus by manual inoculation with a concentrated slurry of virus-infested soil as inoculum. None of the plants developed symptoms. Johnson (12) attempted to transmit the virus in the same way but was not successful.

Seed Transmission Studies -- Seed was collected from 20 varieties of known diseased wheat plants. Fifty seeds of each variety were planted in virus-free soil and the plants were grown to maturity at temperatures averaging 62° F. In two trials no plants developed symptoms at any time. Control plants of the same varieties grown in virus-infested soil developed excellent symptoms.

Fenne and Roane (6) suggested the possibility of seed transmission in Virginia, but McKinney (16), McKinney et al. (28), and Koehler et al. (15) reported no seed transmission. The cumulative evidence would seem to support the latter view.

Insect Transmission Studies -- Several attempts in Kansas to transmit the virus with three aphid species and the eriophyid mite, Aceria tulipae (Keifer), have been negative. Field observations of disease development have not pointed to an aerial vector. McKinney et al. (30) have summarized the evidence concerning vectors. There is no evidence for flying insect vectors; however, evidence is presented for a very small, living root vector which may also serve as an alternate host.

Comparison of Other Characteristics

Incubation Period -- The long incubation periods of up to 10 weeks or more reported by McKinney (19, 25) have been characteristic of the Kansas, Illinois, and Nebraska viruses studied in this work. With all 10 soils studied the incubation period typically varied from 40 to 75 days, the mean being 69.4. The minimum of 40 days occurred occasionally with very susceptible varieties such as MoKing. The longest recorded incubation period was 106 days. This occurred during a very cloudy winter when the light intensities were unusually low and the growth conditions in the greenhouse rather poor. The unknown variety of rye, mentioned previously, which produced good symptoms when grown in infested soil at 70° F (36) unfortunately still required an average minimum incubation period of 67 days before symptoms appeared.

Inactivation of Virus in Soil -- Four virus-infested soils from Kansas were steam-steri-

lized, using standard technics. Pawnee wheat was planted in these soils in 8-inch sterilized clay pots and during growth was kept in a favorable environment for symptom development. In two trials totaling 54 plants, symptoms failed to develop in any plant. Johnson (11) and Koehler et al. (15) also reported virus inactivation in the soil by heat. Inactivation of the virus in the soil by various chemicals, including some insecticides and fumigants, has been reported by McKinney (16), Johnson (12, 13), and McKinney et al. (30).

Depth of Virus Penetration into the Soil -- Three virus-infested fields were chosen. In each a soil area 2 feet square was outlined. Soil from each square was collected carefully in 2-inch layers which were stored in separate containers. The layers collected were as follows: 0-2, 2-4, 4-6, 6-8, and 8-10 inches. Pawnee wheat plants were then grown in the greenhouse in the various soil layers to see whether the virus was present and equally infectious at the different depths. Plants developed excellent symptoms in all soils, indicating that the virus was not only present but also uniformly infectious at all depths tested. Koehler et al. (15) have reported infection from soil layers as deep as 26 inches in Illinois.

Amounts of Infested Soil Necessary for Plant Infection -- Various methods were used to determine approximate amounts of infested Kansas soils necessary for natural infection of susceptible wheat plants. Culms and roots of dead diseased plants still covered with infested soil were added to virus-free soil in small but varying amounts. In five attempts during five seasons 140 Pawnee and MoKing wheat plants were grown in these soils and 25 plants developed symptoms.

In another series of experiments very small amounts of wet virus-infested soil were plastered to wheat seeds before planting and around the roots of three-leaf stage seedlings. These were then planted in non-virus soil. Only one Pawnee wheat plant out of 168 grown during six trials and five seasons developed symptoms. This was one of the transplanted seedlings.

A high percentage of successful infection occurred when soil mixtures contained 10 percent or more of the virus-infested soil. These results were consistent in 12 trials with several hundred Pawnee and MoKing wheat plants. Infection percentages were as high when layers of infested soil were used either above or below virus-free soil as when the two soils were mixed thoroughly.

In three trials various other schemes to obtain natural infection, using very small amounts of infested soil or items contaminated with virus-infested soil, failed consistently. The methods tried were: 1) unwashed pieces of clay pots in which virus-infested soil had been stored were placed in sterilized pots containing noninfested soil; 2) seed was rubbed with dry virus-infested soil prior to planting in virus-free soil; 3) roots of Pawnee and MoKing wheat seedlings were clipped and dipped in a slurry of virus-infested soil for up to 6 hours prior to planting in noninfested soil; 4) varying amounts of straw from dead diseased plants were added to virus-free soil; 5) concentrated fresh diseased wheat plant extract (50 ml) was added to each 8-inch clay pot of virus-free soil after planting; 6) planting and potting tools and hands were dusted with dry virus-infested soil prior to planting wheat in virus-free soil; 7) a virus-infested soil suspension (50 ml) was poured into virus-free soil in 8-inch clay pots planted with susceptible wheat varieties; 8) a soil-free filtrate obtained from virus-infested soil suspensions (50 ml) was poured into each 8-inch pot containing noninfested soil and planted to susceptible wheat varieties.

The results reported above are quite similar to those reported by others working with various virus-infested soils. McKinney (23, 24, 26) could not infest virus-free soil by adding diseased leaf tissue, diseased leaf extracts, clean stubble of diseased plants, or old diseased roots. He did obtain infection, however, by sprinkling infested soil liberally in seed trenches, but obtained much better results when a layer of infested soil 2 to 4 inches deep was added to the virus-free soil. Neither Webb (42) in the United States, nor Ikata and Kawai (9) in Japan, obtained infection from soil filtrates. Webb reported a very low percentage of infection when a small amount of infested soil was present or was added to the filtrates. As the amount of infested soil increased the percentage of plants showing symptoms also increased. With 3 percent infested soil only 5 percent of his plants developed symptoms. Above 12 percent infested soil the incidence of infection increased sharply. Koehler et al. (15) were also able to obtain very low percentages of infection with very small proportions of infested soil, and McKinney et al. (30) reported some successful infections after splash contamination when infested soil splashed over into noninfested soil. Recently McKinney et al. (30) have shown that the addition of fresh (but not old or dead) roots or crowns of diseased plants to noninfested soil

may initiate soil infestation and result in plant infection.

Also, McKinney (27) recently has reported methods of maintaining infectious cultures without the use of soils. Inert materials such as washed sand and gravel have been infested by the addition of fresh young roots and crowns of diseased wheat plants.

Virus Spread in the Soil -- Several attempts were made outdoors to demonstrate the movement of the virus with the movement of surface water from infested to noninfested soil. In one series of experiments conducted for three seasons, greenhouse flats were used. These were 3 feet square, 6 inches deep, and had metal center partitions which eliminated root contact between the two sections. One side of each flat was filled to the top of the partition with infested and the other side with noninfested soil. After planting with Pawnee or MoKing wheat, the virus-infested sides of the flats were raised and the soil was watered regularly on the high side so that some infested soil was washed gradually across the partition onto the surface of the noninfested soil. Control flats were identical except that they were watered throughout with extreme care to avoid splashing and were not tilted. Results in the 20 tilted flats were identical with those in the six horizontal control flats. Plants on the infested side developed symptoms. Those on the noninfested side did not.

In another series of experiments an area of native unplowed sod was tested for infestation and found to be free of the virus. Two area, 75 by 40 feet, one on a hillside having a slope of approximately 10 degrees and one on the level, were prepared for planting. In each plot three holes were dug, each 1 foot deep and 3 feet square. These holes were then filled with known virus-infested soil and the boundaries were marked. Susceptible wheat varieties were planted throughout the two plots in both infested and noninfested areas for four seasons. Some symptoms developed on plants growing in the infested areas each year, even though 2 of the years, 1955 and 1956, had poor conditions for symptom development. Symptoms developed on plants outside the infested areas in only the second season, 1954, which happened to be an excellent year for symptom development throughout eastern Kansas. Eight plants showing symptoms were seen in the hillside plot. These were all downhill from the infested area but none were more than 10 inches away from it. In the level plot six plants outside of the infested area developed symptoms and all were within 6 inches of the infested area except one which was 18 inches away. In both plots most plants near the infested areas remained symptomless. According to these data, it appeared that under Kansas conditions the virus moved downhill by soil and water very slowly if at all.

Since the few diseased plants growing in originally noninfested soil were close to the infested areas, there was the possibility that they were infected via roots which extended into the infested soils. To check this possibility two series of experiments were performed. In the first, susceptible winter wheat varieties were grown outdoors in buried greenhouse flats 8 inches deep, containing a 1-inch layer of infested soil on the bottom, the remainder of the soil on top being noninfested. The seeds were planted in the top layer so that it would be necessary for rootlets to grow gradually into the bottom infested layer. Seventy-nine of 86 plants developed good symptoms under these conditions during 2 years. In the second experiment a metal center partition was placed in each deep flat. Infested soil was placed on one side of this partition, noninfested soil on the other, and the susceptible wheat was planted only on the noninfested side. After emergence of the wheat, the partition was raised 1 inch so that roots could cross from the noninfested to the infested area, but only at the bottom of the flat. Twenty-eight of 94 plants in these flats developed symptoms during 2 years. Both experiments indicated that plants could become infected readily via roots extending into infested from noninfested areas.

These results concerning root penetration of infested soil agree with those of McKinney (17). However, he also was convinced that the causal agent was spread at least slightly by the movement of soil water (16). Field observations in Kansas would agree with his position but the experiments just described concerning the tilted flats and hillside plots do not. Of course, the environment in Illinois and the eastern United States is typically much more favorable than Kansas conditions for severe disease development, and this in itself may account for the differences. If the Kansas experiments could have been extended over a longer period of time and during a series of wet years, it might have been possible to demonstrate more rapid development of soil infestation by the surface movement of infested soil and water. Again, the generally poorer results in Kansas may be due to some unknown antagonism which limited spread of the virus in the soils tested. However, based upon field observations, there is no doubt that the virus has spread and is spreading by some means in Kansas soils.

Comparison of Losses and Control Measures

Losses from soil-borne wheat mosaic virus have been severe. McKinney et al. (28) reported losses of 40 percent in Illinois, and, according to Koehler et al. (15), "If resistant varieties had not been found, wheat would not be grown in some areas of Illinois." In Virginia, Roane et al. (33) reported yields of 49 bushels per acre on noninfested soil and 13.5 bushels per acre on comparable virus-infested soil. Bretz (4) in Missouri reported losses of 50 percent in some fields. No losses consistently so great as these have been reported in Kansas. In 1952 losses varied from 8 to 13 percent in diseased fields (5), in 1954 they were estimated at 26 percent (8), and in 1957 at about 16 percent (37). In other years since 1951 the losses have been much less. Although percentage losses are not so great in Kansas, the infested acreage is so large, nearly 400,000 acres in 1957, that this disease must be considered an important wheat disease in the State. Probably the losses would be far greater and more comparable with those occurring in Illinois and eastward if the environment were typically more favorable for disease development.

An effort was made to control the disease, or at least to reduce its severity, by the addition of complete fertilizers in the field and complete nutrient solutions in the greenhouse. Soluble iron, magnesium, and nitrogen were added separately in optimum amounts in other experiments, both in the field and greenhouse. No treatments reduced symptom severity in any case when compared with untreated controls. In fact, symptoms were often more pronounced in plants given optimal nutrition. Also, it should be noted that the most fertile infested fields have often been damaged most severely in Kansas. These results are very similar to those reported by workers in several States (10, 15, 16, 40).

Resistant varieties when utilized have provided satisfactory control in all areas where this disease is known. Beginning in the 1951-52 season varietal test plots have been planted each year in various virus-infested soils in eastern Kansas. By 1957, 25 plots, each one averaging from 80 to 100 varieties, had been planted. These varietal reactions and some other experiments will be reported in detail in a separate paper with Hurley Fellows[3], but in general the results in both greenhouse and field indicate that those wheat varieties which have been resistant or susceptible in the infested soils of Illinois (3, 15, 43), Virginia (33), the Carolinas (32), and Oklahoma (44) have also had the same general reaction pattern in Kansas. There is only one conspicuous exception, mentioned previously. The very susceptible rosetting wheats such as Harvest Queen (susceptible), MoKing, Stafford, Bison, Kiowa, and Purdue 45-1834, although tested repeatedly, have never developed rosette in infested Kansas soils, even though they have been among the most susceptible varieties tested. The highly resistant varieties Concho and Comanche are now recommended for use in infested Kansas soils.

DISCUSSION

Comparison of United States Viruses -- A comparison of known characteristics of soil-borne wheat mosaic viruses of various parts of the United States shows remarkable similarities in most respects. The evidence would indicate that the viruses typically enter the host from infested soils through the roots and root crowns. There are no known aerial means of spread in the field, and all evidence to date would indicate that no aerial insect or mite vectors exist. However, the circumstantial evidence is strong for a very small living soil- or root-inhabiting vector, possibly an alternate host. It is also possible to infest non-virus soils by the addition of relatively low percentages of virus-infested soils. None of the viruses is transmitted by seed. Favorable environmental factors, such as temperature, soil moisture, and day length, are essentially identical, as are the optimum epidemiological factors for disease development in the field.

Field symptoms as described are very similar throughout the United States wherever the soil-borne wheat mosaics have been found, that is in a region including several of the southeastern and north-central States and certainly extending as far west as central Kansas, Oklahoma, and Nebraska. Individual plant symptoms in the United States are also quite similar except that the rosette reaction, characteristic of a few very susceptible varieties in Illinois and eastward, has not been reported from Kansas, Oklahoma, or Nebraska. Based upon symptoms, at least two virus strains have been described in the eastern United States, but no sure evidence for strain differences has been seen in Kansas. Both yellow and green symptoms

[3]Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, located at the Kansas Agricultural Experiment Station, Manhattan.

occur but thus far they have not been separated by any method utilized. Apparently the development of different individual plant symptoms has not depended upon the strain present but for the most part upon the susceptibility or reaction of the wheat variety, the level of virus infestation in a particular soil, and the environment. Symptom differences between various wheat varieties and in different environments have been great, but in a constant environment specific wheat varieties have given identical symptoms when grown in all virus-infested soils tested in the greenhouse and also in about 25 experimental field plots grown in Kansas. If two strains are present, it would appear then that they are both distributed in all soils tested, the yellow and the green strains being intermixed. Perhaps the apparent absence of the rosette reaction from Kansas soils also indicates the absence of the green strain which seemed to be closely associated with wheat rosette as reported by McKinney (21).

All host ranges reported have been similar and very narrow, including wheat, rye, and sometimes barley. One grass host, Bromus commutatus Schrad., has also been reported in eastern United States, but no infected native grasses have been reported previously in the Great Plains. In 1958, however, an unidentified species of annual brome grass, probably either Bromus tectorum L. or Bromus japonicus Thunb., which showed mosaic-like symptoms when growing at the border of a badly diseased Kansas wheat field, was seen. These plants probably were infected with the virus but there is no definite proof of this as yet.

All viruses tested in the United States have been manually transmissible with difficulty. Typically only a small percentage of inoculated plants have developed symptoms, although recently a method has been developed which results in a higher percentage of infection (30). All viruses studied have exhibited a long incubation period, averaging about 70 days. Inactivation of all virus-infested soils has been accomplished by steam sterilization and various insecticides and fumigants.

The evidence presented would certainly seem to indicate that the various soil-borne wheat mosaic viruses reported in the United States are probably strains of the same virus.

Japanese Soil-Borne Wheat Mosaic Viruses -- The yellow and green soil-borne wheat viruses of Japan share several common characteristics with the soil-borne wheat mosaic viruses of the United States. They enter the host only through the roots and root crowns. They need essentially the same temperatures for symptom expression and are not induced by infested soil filtrates being poured on virus-free soil (9). Resistance appears to be a simple monogenic Mendelian dominant and hence it has been easy to develop Japanese wheats resistant to the disease (31). This has also been the common experience of workers seeking resistance in the United States.

Wada and Hukano (38, 39) reported X-bodies, as had been reported in the United States, but there were two types, one characteristic of the yellow mosaic and one of the green. Both of these types were observed in cells of plants infected by a combination of the viruses as well as a new intermediate X-body type. These workers reported the testing of 30 virus-infested soils. Eight of these produced green mosaic only, 4 yellow mosaic only, and 18 produced both. Separate genes for resistance to the green and yellow mosaics were also reported[4]. These data were presented as evidence that two strains or possibly two viruses were involved.

It is certainly clear that the soil-borne wheat viruses of Japan and the United States are similar in some respects, and it is probably not certain yet that they differ markedly in any important characteristics. However, until more data are available, it would be premature to consider the Japanese viruses as essentially the same as those in the United States. It is not unlikely, though, that they all may eventually be shown to be strains of the same virus.

Soil-Borne Oat Mosaic Virus -- The soil-borne oat mosaic viruses described by Atkinson (2) and McKinney (22), occurring in the southeastern States (25) have not been found in Kansas or reported from the Plains States. These viruses appear so far to be similar to the soil-borne wheat mosaic viruses in most respects studied except host range and certain symptom patterns (22, 29). Similarities include general epidemiology, manual transmissibility with difficulty, and some symptom similarity, including the rosette reaction in a few oat varieties and hybrids. Soil infestation characteristics seem to be similar. Neither virus is seed-borne, and elimination of both viruses from the soil as infective agents has been reported by similar physical and chemical methods (22, 29). The oat virus thus far has infected only oats and closely related species of Avena and has not infected wheat in the field (2, 22). Although

[4]This information obtained through conversations with E. G. Heyne of the Kansas Agricultural Experiment Station after his trip to Japan. Original literature was not available.

probably very closely related to soil-borne wheat mosaic virus, this virus (or viruses) at the moment must be considered as distinct.

Literature Cited

1. ATKINSON, R. E. 1945. Mosaic of wheat in the Carolinas. Plant Disease Reptr. 29: 86.
2. ATKINSON, R. E. 1945. A new mosaic chlorosis of oats in the Carolinas. Plant Disease Reptr. 29: 86-89.
3. BEVER, WAYNE M., and J. W. PENDLETON. 1954. The effect of soil-borne wheat mosaic on yield of winter wheat. Plant Disease Reptr. 38: 266-269.
4. BRETZ, T. W. 1944. Wheat mosaic survey in Illinois and Missouri and notes on other wheat diseases. Plant Disease Reptr. 28: 418-419.
5. FELLOWS, H., W. H. SILL, Jr., and C. L. KING. 1953. The 1952 epiphytotic of a soil-borne wheat mosaic in Kansas. Plant Disease Reptr. 37: 287-289.
6. FENNE, S. B., and C. W. ROANE. 1952. Wheat mosaic in Virginia. Plant Disease Reptr. 36: 212.
7. HANSING, E. D., W. H. SILL, Jr., C. O. JOHNSTON, and C. T. ROGERSON. Kansas phytopathological notes: 1956. Trans. Kansas Acad. Sci. (In Press)
8. HASKETT, W. C., W. H. SILL, Jr., C. O. JOHNSTON, E. D. HANSING, and HURLEY FELLOWS. 1956. Kansas phytopathological notes for 1954. Trans. Kansas Acad. Sci. 59: 51-56.
9. IKATA, S., and I. KAWAI. 1937. Some experiments concerning the development of yellow mosaic disease (white streak) of wheat. Relation between the development of yellow mosaic disease of wheat and soil temperature. Jour. Plant Prot. 24: 491-501, 847-854. (In Japanese) (Abstracted in Rev. Appl. Mycol. 18: 98. 1939)
10. JOHNSON, A. G., H. H. McKINNEY, R. W. WEBB, and C. E. LEIGHTY. 1924. The rosette disease of wheat and its control. U. S. Dept. Agr. Farmers' Bul. 1414.
11. JOHNSON, F. 1942. Thermal inactivation of wheat mosaic virus in soils. Science (n. s.) 95: 610.
12. JOHNSON, F. 1945. Epiphytology of winter wheat mosaic. Ohio Jour. Sci. 45: 85-96.
13. JOHNSON, F. 1945. The effect of chemical soil treatments on the development of wheat mosaic. Ohio Jour. Sci. 45: 125-128.
14. JOHNSTON, C. O., E. D. HANSING, W. H. SILL, Jr., W. C. HASKETT, and C. T. ROGERSON. 1957. Kansas phytopathological notes: 1955. Trans. Kansas Acad. Sci 60: 148-154.
15. KOEHLER, BENJAMIN, W. M. BEVER, and O. T. BONNETT. 1952. Soil-borne wheat mosaic. Illinois Agr. Exp. Sta. Bul. 556: 567-599.
16. McKINNEY, H. H. 1923. Investigations of the rosette disease of wheat and its control. Jour. Agr. Res. 23: 771-800.
17. McKINNEY, H. H. 1925. A mosaic disease of winter wheat and winter rye. U. S. Dept. Agr. Bul. 1361.
18. McKINNEY, H. H. 1930. A mosaic of wheat transmissible to all cereal species in the tribe Hordeae. Jour. Agr. Res. 40: 547-556.
19. McKINNEY, H. H. 1931. Differentiation of viruses causing green and yellow mosaics of wheat. Science 73: 650-651.
20. McKINNEY, H. H. 1937. Mosaic diseases of wheat and related cereals. U. S. Dept. Agr. Circ. 442.
21. McKINNEY, H. H. 1944. Descriptions and revisions of several species of viruses in the genera Marmor, Fractilinea and Galla. Jour. Wash. Acad. Sci. 34: 322-329.
22. McKINNEY, H. H. 1946. Mosaics of winter oats induced by soil-borne viruses. Phytopathology 36: 359-369.

23. McKINNEY, H. H. 1946. Soil factors in relation to incidence and symptom expression of virus diseases. Soil Science 61: 93-100.
24. McKINNEY, H. H. 1948. Wheats immune from soil-borne mosaic viruses in the field, susceptible when inoculated manually. Phytopathology 38: 1003-1013.
25. McKINNEY, H. H. 1953. Virus diseases of cereal crops. U. S. Dept. Agr. Yearbook. pp. 350-360.
26. McKINNEY, H. H. 1953. Soil-borne wheat mosaic viruses in the Great Plains. Plant Disease Reptr. 37: 24-26.
27. McKINNEY, H. H. 1957. Maintenance of naturally infectious cultures of the soil-borne viruses of wheat mosaic and of oat mosaic, without the use of soil. Plant Disease Reptr. 41: 254-255.
28. McKINNEY, H. H., R. W. WEBB, and G. H. DUNGAN. 1925. Wheat rosette and its control. Illinois Agr. Exp. Sta. Bul. 264.
29. McKINNEY, H. H., T. R. STANTON, J. L. SEAL, T. H. ROGERS, W. R. PADEN, G. K. MIDDLETON, and U. R. GORE. 1949. Mosaics of winter oats and their control in the southeastern States. U. S. Dept. Agr. Circ. 809.
30. McKINNEY, H. H., W. R. PADEN, and BENJAMIN KOEHLER. 1957. Studies in chemical control and overseasoning of, and natural inoculation with, the soil-borne viruses of wheat and oats. Plant Disease Reptr. 41: 256-266.
31. MIYAKE, M. 1939. Mendelian inheritance of the resistance against the virus disease in wheat strains. Japanese Jour. Genet. 14: 239-242. (Abst. in Rev. Appl. Mycol. 19: 650. 1940)
32. MOSEMAN, J. G., H. H. McKINNEY, and C. W. ROANE. 1954. Reaction of wheat varieties and selections to the soil-borne viruses in the southeastern United States. Plant Disease Reptr. 38: 19-24.
33. ROANE, C. W., T. M. STANLEY, and H. H. McKINNEY. 1954. Observations on wheat mosaic in Virginia. Plant Disease Reptr. 38: 14-18.
34. ROANE, C. W., and S. B. FENNE. 1955. Some new plant disease records for Virginia. Plant Disease Reptr. 39: 695-696.
35. SILL, W. H. Jr., W. C. HASKETT, H. FELLOWS, E. D. HANSING, and C. O. JOHNSTON. 1954. Kansas Phytopathological notes: 1953. Trans. Kansas Acad. Sci. 57: 453-457.
36. SILL, W. H. Jr. 1955. A winter rye useful in identifying soil-borne wheat mosaic virus. Phytopathology 45: 637.
37. SILL, W. H. Jr., and CLAUDE L. KING. 1958. The 1957 soil-borne wheat mosaic epiphytotic in Kansas. Plant Disease Reptr. 42: 513-516.
38. WADA, E., and H. HUKANO. 1934. On the difference of X-bodies in green and yellow mosaic of wheat. Agr. and Hort. 9: 1778-1790. (Japanese with English Summary.) (Abst. in Rev. Appl. Mycol. 14: 618. 1935.)
39. WADA, E., and H. HUKANO. 1937. On the difference and discrimination of wheat mosaics in Japan. Jour. Imp. Agr. Exp. Sta. 3: 93-128. (Japanese with English summary.) (Abst. in Rev. Appl. Mycol. 16: 665. 1937.)
40. WADSWORTH, D. F., and H. C. YOUNG, Jr. 1953. A soil-borne wheat mosaic virus in Oklahoma. Plant Disease Reptr. 37: 27-29.
41. WEBB, R. W. 1927. Soil factors influencing the development of the mosaic disease in winter wheat. Jour. Agr. Res. 35: 587-614.
42. WEBB, R. W. 1928. Further studies on the soil relationships of the mosaic disease of winter wheat. Jour. Agr. Res. 36: 53-75.
43. WEBB, R. W., C. E. LEIGHTY, G. H. DUNGAN, and J. B. KENDRICK. 1923. Varietal resistance in winter wheat to the rosette disease. Jour. Agr. Res. 26: 261-270.
44. YOUNG, H. C., Jr. (Oklahoma State University, Stillwater, private conversations and correspondence.)

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, KANSAS STATE COLLEGE, MANHATTAN, KANSAS

DIFFERENCES IN HOST REACTIONS TO K&S AND WESTERN X-DISEASE VIRUSES

E. L. Reeves and Philip W. Cheney[1]

A virus naturally occurring but symptomless in Kwanzan and Shiro-fugen varieties of flowering cherries was reported and shown to produce fruit symptoms of the little cherry-type on sweet cherry (2). No definite name was given the virus since its possible relationship to other little cherry-type viruses was not established. Temporarily, the virus is called "K&S" for the first letters of Kwanzan and Shiro-fugen. Four additional varieties of flowering cherries (Amanogawa, Fugenzo, Taki-nioi, and Autumnalis) were subsequently found naturally carrying the virus; they also are normal in appearance and growth.

The K&S virus produces little cherry-type fruit symptoms on sweet cherry suggestive of the little cherry disease occurring in the Kootenay area of British Columbia (2). Sweet cherry trees infected with X-disease virus also produce small fruits similar in some respects to the little cherry-type fruits produced on K&S-virus-infected trees. Since 1953, numerous comparisons of different hosts infected with K&S and western X-disease viruses have been made. The writers, working mostly with peach, chokecherry, and sweet cherry, obtained information indicating that the so-called K&S virus differs from western X-disease virus. It is the purpose of this paper to report the results of these tests.

Some differences recognized thus far are conspicuous in certain species, while other dissimilarities can best be evaluated by studying the sequence of symptom development for several years. Symptoms are discussed under the hosts tested.

PEACH: Symptoms caused by the western X-disease virus in peach are well known and documented (1). Inoculum taken from sweet and flowering cherries carrying K&S virus and placed in peach has resulted in no recognizable symptoms. Eleven peach trees 3 and 4 years old and 113 seedling nursery trees were used in the tests. Attempts to recover the K&S virus from peach trees inoculated in 1955 have been unsuccessful.

CHOKECHERRY: Twenty-three chokecherry plants including Prunus virginiana and P. virginiana var. demissa have been inoculated with sweet cherry material carrying the K&S virus. No recognizable symptoms appeared on any of the chokecherry plants. One attempt to recover K&S virus from chokecherry gave negative results. Symptoms in chokecherry caused by western X-disease virus are well known and were previously described (1).

SWEET CHERRY: Since 1949 more than 300 sweet cherry trees of 15 varieties have been inoculated with K&S virus and have expressed symptoms. The small fruits produced on K&S-virus-infected trees resemble in some respects those borne on similar trees infected with western-X-disease virus. Symptoms expressed by and reactions of sweet cherry trees separately infected by these viruses are as follows:

a. Rootstock differences.

K&S virus:-- Fruit symptoms expressed equally well by infected trees on mazzard or mahaleb rootstock.

Western X-disease virus: -- Fruit symptoms expressed by infected trees on mazzard rootstock; infected trees on mahaleb often wilt or decline and develop poor fruit symptoms if the tree survives.

b. Fruit symptoms in years following infection.

K&S virus:-- Some variations occur in fruit symptoms on different varieties. Lambert is used as a typical example. The first year after infection, most fruits showing symptoms are about 1/2 normal size, pinkish-red at harvest time, pointed and somewhat angular. The second year after infection most affected fruits are 2/3 to 3/4 normal size, pointed and more angular, and some fruits are nearly 3-sided. Usually a few 1/2-size pinkish-red fruits are present the second year, and there are seldom any thereafter. Three years or more after fruit symptoms first appear, fruits are often 3/4 to 4/5 normal size but have less sugar than normal ones and lack flavor.

[1] Respectively, Pathologist, and Assistant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Wenatchee, Washington.

These fruits also are dull red.

Western X-disease virus:-- Fruits that show symptoms are small, averaging about 1/2 normal size, and pinkish-red; general appearance and size are much like those of fruits on K&S-virus-infected trees the first year after infection. Size and color of fruits that show symptoms are fairly constant in succeeding years. Affected fruits are somewhat bitter.

c. Time of appearance of fruit symptoms after inoculation of bearing tree.

K&S virus:-- Fruit symptoms usually apparent within 1 year after inoculation if there are no complicating weather or growing conditions.

Western X-disease virus:-- Few trees exhibit recognizable fruit symptoms the first year after inoculation; 2 or 3 years and sometimes longer elapse between inoculation and appearance of fruit symptoms.

d. Distribution on tree of fruits that show symptoms.

K&S virus:-- The first year after infection, fruits showing symptoms are usually relatively near points of inoculation, with only a scattering of a few fruits with symptoms on other branches. Fruits with symptoms are well distributed over tree after the first year.

Western X-disease virus:-- Fruits showing symptoms are usually confined for 2 years or longer to the branch receiving inoculum; then several years may elapse before branches not receiving inoculum bear fruits that express symptoms.

e. Transmissibility.

K&S virus:-- Easily transmitted. Usually 100 percent of attempted transmissions are successful when inoculum is taken from any part of tree infected 2 years or longer, indicating that the virus is fully systemic in host.

Western X-disease virus:-- Difficult to transmit. Seldom more than 50 percent of attempted transmissions, and often less than 10 percent, are successful. Complete failures are common unless inoculum is carefully selected from branches bearing fruits that show symptoms, which indicates that the virus is not fully systemic.

f. Foliage symptoms.

K&S virus:-- A detailed report of the foliage symptoms on sweet cherry infected with K&S virus has been published (3). Autumnal yellow and bronze coloration starts to develop on certain varieties during late July or early August during some seasons or in September if weather conditions are less favorable for color development. Interveinal areas are reddish-bronze; veins and basal portion of leaves are mostly green without red pigmentation. Deacon, Sam, Bing, Star, Van, and Black Tartarian varieties express distinctive foliage coloration; Deacon develops the earliest and most pronounced foliage symptoms. Lambert develops the least coloration and is considered a poor test variety for expression of foliage symptoms.

Western X-disease virus:-- In Washington, trees infected for many years may have foliage with variable coloration. Some leaves may appear severely sunburned or yellowish-green, others are light brown or bronzed, and still others may be nearly normal in appearance or dull green. Cherry trees infected for only 3 or 4 years usually show no foliage symptoms.

The conclusion that K&S virus is different from western X-disease virus is primarily based on the following points: 1) peach and chokecherry exhibit distinctive symptoms when infected with western X-disease virus but have not been successfully infected with K&S virus; 2) symptoms expressed by and reactions of sweet cherry trees separately infected by these viruses differ in several respects, particularly as to the foliage symptoms expressed and the ease with which the viruses are transmitted.

Literature Cited

1. REEVES, E. L., EARLE C. BLODGETT, T. B. LOTT, J. A. MILBRATH, B. L. RICHARDS, and S. M. ZELLER. 1951. Western X-disease. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr., Agr. Handb. 10, pp. 43-52, illus.

2. REEVES, E. L., PHILIP W. CHENEY, and J. A. MILBRATH. 1955. Normal-appearing Kwanzan and Shiro-fugen oriental flowering cherries found to carry a virus of little cherry type. Plant Disease Reptr. 39: 725-726.
3. REEVES, E. L., and PHILIP W. CHENEY. 1956. Foliage symptoms on sweet cherry infected with the little-cherry-type virus obtained from Kwanzan and Shiro-fugen oriental flowering cherries. Plant Disease Reptr. 40: 408-409.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

NURSERY BEHAVIOR OF INFECTED WEST INDIAN LIME SCIONS ON TRISTEZA TOLERANT AND INTOLERANT ROOTSTOCKS[1]

D. C. Giacometti[2] and A. S. Costa[3]

Summary

West Indian lime scions infected with three mild and one severe sources of the tristeza virus were compared in nursery rows at Rio de Janeiro, Brazil. Measurements taken 6 months after budding indicated that the scions infected with the mild virus sources made better growth on sour orange or Florida rough lemon rootstocks than scions infected with the severe source of the virus.

Symptoms similar to seedling yellows were noted on axillary growth made by the sour orange rootstock of plants that had a scion infected with the severe virus source.

Studies on citrus scion-rootstock interrelationships with combinations affected by the tristeza disease have been conducted in various countries (1, 5). It has been shown that the use of tristeza-tolerant rootstocks offers adequate commercial control for the disease when the scions are sweet orange, tangerines or mandarins, and a few other types that possess tolerant tissues.

The use of tolerant rootstocks alone is not entirely satisfactory in the case of tops such as grapefruit, West Indian limes, and some other types with intolerant tissues. The injury caused by the disease to these scions on tolerant rootstocks might be severe enough to make the combination commercially unprofitable.

Two main lines of attack seem more promising for development of tristeza control measures in the case of combinations with intolerant tops: (a) the selection of types or mutants with tissues somewhat more tolerant; (b) the use of propagative material that naturally or artificially is infected with mild strains of the virus, assuming that a certain degree of protection is thus secured.

The existence of mild and severe forms of the tristeza disease, first indicated from Africa (1, 10) was later studied in Brazil and in the United States (6, 9). It was demonstrated that the mild and severe forms of the disease were associated with strains of the virus. The possibility that mild strains of the virus may offer protection against invasion by and establishment of severe strains became thus an important point to be determined, and experiments aiming at elucidating this problem were started at Campinas, Brazil (3).

The degree of protection that may be offered by mild strains against invasion by severe strains depends not only on the plant itself and on the mild strain used to premunize the plant, but also on the natural virus strain population occurring in different areas. This problem is therefore one that has to be studied finally in the areas concerned. It was thus thought to be of interest to compare the behavior of West Indian lime scions invaded by mild and severe strains of the tristeza virus under the conditions prevailing in the citrus-growing areas of the state of Rio de Janeiro. The present paper reports the observations made in the nursery.

MATERIAL AND METHODS

Seeds from two parent trees that represented the West Indian lime clones No. 7 and No. 32 were sown and the seedlings obtained were used in the present experiment. Two seedlings from each source were budded separately with buds carrying virus from the following sources: (a) the mild virus from parent tree No. 7; (b) the mild virus from parent tree No. 32; (c) virus sources No. 83 and No. 84, received from the Instituto Agronômico, Campinas, representing a mild and a severe type, respectively.

Typical symptoms of veinal flecking were observed 6 months after inoculation on those seedlings that had been inoculated with virus from sources No. 83 and No. 84. Those inocu-

---

[1]Project sponsored by the Conselho Nacional de Pesquisas (Brazilian National Research Council).

[2]Horticulturist, Instituto de Ecologia e Experimentação Agrícolas, Brazilian Ministry of Agriculture.

[3]Pathologist, Instituto Agronômico, Campinas, Brazil.

lated with virus from the two local mild sources did not show any noticeable symptoms.

Buds from the infected seedlings were then budded on sour orange and Florida rough lemon rootstocks, 16 and 8 months old, respectively, in the nursery. From each seedling a total of ten buds was taken, five being budded on each type of rootstock. Virus-free buds from each of the two sources were also budded on the two rootstocks as controls. Two months after budding the rootstock tops were cut back and the West Indian lime buds were permitted to grow, as well as the rootstock axillary sprouts.

RESULTS

Growth measurements and symptom observations recorded 6 months after budding are given in Table 1.

Table 1. Growth measurements of West Indian lime scions infected with mild and severe strains of the tristeza virus on sour orange and on Florida rough lemon rootstock.

Virus source	Average[a] growth of scion when budded on the indicated rootstock (centimeters)	
	Sour orange	Florida rough lemon
Check	65.3	43.3
Mild 7	62.1	38.8
Mild 32	67.0	58.2
Mild 83	52.7	41.6
Severe 84	37.2	35.0

[a]Average based on scion growth of 10 plants for the first three sources; on 15 plants for the fourth; on 20, for the fifth.

They indicate that the growth made by the tops infected by virus from the local mild sources was better than that of the tops infected by virus from the two sources received from Campinas, when the rootstock was the sour orange. Thus, the scion growth from buds carrying virus from sources No. 83 and No. 84 was stunted and showed yellowing and stem-pitting symptoms. Also, the axillary branches of the sour orange rootstock showed top yellowing (Fig. 1), the symptoms being similar to those described for seedling yellows (4). The sour orange rootstocks budded with West Indian lime infected by virus from sources No. 7 and No. 32 developed few axillary branches and these were normally green. The scions made excellent growth (Fig. 2) when compared with that of scions infected by virus from severe sources.

On the Florida rough lemon rootstocks, the West Indian lime buds carrying the three mild viruses did not show much difference in growth, but growth of these was better than that made by the buds infected from the severe virus source. It was also noticed that the type of growth made by scions infected by virus from source No. 84 was bushy in appearance. This was also true, although to a lesser extent, for the growth of scions infected by virus from source No. 83. On the other hand, the tops carrying virus from sources No. 7 and No. 32 were normal and presented only mild stem-pitting symptoms.

FIGURE 1. West Indian lime cion infected by a severe virus from source No. 84) on a sour orange ootstock. The axillary branches of ıe rootstock show symptoms of seed- ing yellows.

FIGURE 2. West Indian lime scion infected by a mild virus source (No. 7) on a sour orange rootstock. Scion growth is very good when compared with that depicted in Figure 1.

The apparently smaller growth made by the scions on Florida rough lemon, a tristeza-tolerant rootstock, when compared with that made on sour orange which is intolerant, is to be attributed to the greater age of the latter when they were budded.

Literature Cited

1. BITTERS, W. P., and E. R. PARKER. 1952. Quick decline studies. Rootstock investigations test various top-root combinations in search of tolerant citrus varieties. California Agric. 6: 10-11.
2. COSTA, A. S., T. J. GRANT, and S. MOREIRA. 1954. Reação da laranja azêda à tristeza. Bragantia 13: 199-216.
3. COSTA, A. S., T. J. GRANT, and S. MOREIRA. 1954. Behavior of various citrus rootstock-scion combinations following inoculation with mild and severe strains of tristeza virus. Proc. Florida State Hort. Soc. 67: 26-30.
4. FRASER, L. 1952. Seedling yellows, an unreported virus disease of citrus. Agr. Gaz. N. S. Wales 63: 125-131.
5. GRANT, T. J., and A. S. COSTA. 1948. A progress report on studies of tristeza of citrus in Brazil. Proc. Florida State Hort. Soc. 61: 20-33.
6. GRANT, T. J., and A. S. COSTA. 1951. A mild strain of the tristeza virus of citrus. Phytopathology 41: 114-122.
7. HUGHES, W. A., and C. A. LISTER. 1949. Lime disease in the Gold Coast. Nature 164: 880.
8. McCLEAN, A. P. D., and J. E. van der PLANK. 1955. The role of seedling yellows and stem-pitting in tristeza of citrus. Phyto-pathology 45: 222-224.
9. OLSON, E. O. 1956. Mild and severe strains of tristeza virus in Texas citrus. Phytopathology 46: 336-341.
10. OBERHOLZER, P. C. J. 1947. The bitter-seville rootstock problem. Farming in South Africa 22: 489-495.

TOBACCO RINGSPOT VIRUS ON FLORISTS' HYDRANGEA[1]

C. W. Anderson

During a recent study of the sources of central Florida pepper viruses, a ringspot virus was obtained from Hydrangea macrophylla (Thunb.) DC. In 1957 Brierley and Lorentz (2) described hydrangea ringspot virus from plants of this species, while Brierley (1) obtained tomato ringspot virus from hydrangea in 1954. This report describes the results of tests which proved the Florida hydrangea virus to be related to tobacco ringspot virus.

MATERIALS AND METHODS

Sap inoculation was used exclusively. The viruses tested were: Volusia County hydrangea virus (VHV); tomato ringspot virus (TomRSV) supplied by P. Brierley; and the tobacco ringspot (TRSV) and potato X (PVX) viruses supplied by M. K. Corbett.

RESULTS

Immunological Studies: VHV did not protect tobacco (Nicotiana tabacum L.) plants against TomRSV, while TomRSV failed to immunize tobacco plants against VHV in reciprocal tests. Contrastingly, tobacco plants recovered from VHV were almost 100% immune to TRSV, and vice versa. Therefore, VHV is related to tobacco ringspot virus but not to tomato ringspot virus.

Host Reactions: Table 1 summarizes results obtained in attempts to transmit VHV, TRSV, and TomRSV to various indicator plants. TomRSV differed from the other viruses in its ability to induce mottle on plants of all seven cowpea (Vigna sinensis (Torner) Savi) varieties and in its failure to infect Nicotiana glutinosa L. TRSV killed Catjang cowpea plants or induced severe systemic necrosis and stunt and infected plants of the California Wonder and Tabasco peppers (Capsicum annuum L. and C. frutescens L.). The mottle reaction of Catjang plants infected with VHV resembled that of Catjang plants infected with TomRSV, while VHV did not infect pepper plants. These results indicate that VHV is not identical with Corbett's TRSV isolate.

Large gray lesions appeared on indigo (Indigofera hirsuta L.) leaves inoculated with any one of the three viruses. Lesions appeared on inoculated leaves of Cassia occidentalis L. plants in 2 to 6 days. These lesions were numerous, solid, and tan to reddish-brown. Although they enlarged in size for a number of days, no evidence of systemic infection was obtained. Relatively few blackish, often ringlike lesions formed on inoculated leaves of Cassia tora L. In other tests numerous small necrotic lesions appeared on leaves of Cassia tora or C. occidentalis inoculated with PVX, but no evidence of infection was obtained when indigo plants were inoculated with PVX. PVX incited black lesions on inoculated C. tora leaves and brown ones on C. occidentalis, but in both cases the small solid PVX lesions were distinguishable from those of TRSV and TomRSV.

DISCUSSION AND CONCLUSIONS

This study is believed to constitute the first report of natural infection of hydrangea by TRSV. Leaves of the hydrangea plant from which VHV was obtained were unevenly chlorotic, crinkled, and stunted. The hydrangea and tomato ringspot viruses incite variable symptoms on hydrangea plants (1, 2), but ring patterns are sometimes present. Although ring patterns were lacking in the Volusia material, present evidence does not prove that TRSV can be distinguished from TomRSV or hydrangea ringspot virus by visual inspection of infected hydrangea plants, nor does the evidence establish a causal relationship between TRSV and the symptoms noted in the Volusia-hydrangeas.

In general, the host reactions (Table 1) are typical of those reported for TRSV and TomRSV by other workers. The writer is not aware that other workers tested indigo or Cassia. The susceptibility of peppers to TRSV and the immunity of Nicotiana glutinosa and tomato (Lycopersicon esculentum Mill.) to TomRSV are unusual. Pepper sap diluted 1/50 appeared more infectious than undiluted sap, and transmission of TRSV from pepper to Cassia occidentalis was not obtained, although indirect transmission (pepper to tobacco to Cassia was repeatedly successful.

[1] Florida Agricultural Experiment Station Journal Series, No. 760.

Table 1. The reactions of three ringspot viruses on various indicator plants.

Plant species and variety	Virus		
	TRSV	VHV	TomRSV
Indigofera hirsuta	L[a]	L	L
Cassia occidentalis	L	L	L
Cassia tora	L	L	L
Vigna sinensis: Paraguay #1	N	N	M
Vigna sinensis: Blackeye	N	N	M
Vigna sinensis: Yardlong Bean	N	N	M
Vigna sinensis: Catjang	N	M	M
Vigna sinensis: White Acre Pea	L/N	L?	M
Vigna sinensis: Sill & Walker	L/N	I	M
Vigna sinensis: Black Hog Pea	L/N	L/N	M
Nicotiana glutinosa	R	R	I
Nicotiana tabacum: White Burley & Turkish	R	R	R
Capsicum annuum: California Wonder	X	I	I
Capsicum frutescens: Tabasco	Y	I	Z
Lycopersicon esculentum: Marglobe		I	I
Datura stramonium		M	
Zinnia elegans		M	
Cucumis sativus		M	

[a] The meanings of the symbols are as follows: L = lesions on inoculated leaves only; N = severe necrosis of both inoculated and noninoculated leaves, usually accompanied by death of plant; M = mottle of noninoculated leaves (often accompanied by chlorotic or necrotic lesions on inoculated leaves, and sometimes by some systemic necrosis); R = local and systemic necrotic ringspot disease; X = mild ring lesions inoculated leaves of 1 plant plus recovery from noninoculated leaves of 2 plants; Y = mild ringspots on inoculated and noninoculated leaves of 1 plant plus recovery from noninoculated leaves of 2 plants; Z = mild chlorotic rings on 4 plants but no recovery trials successful; I = immune (no symptoms and all recovery trials from noninoculated leaves negative); / = or; ? = symptoms not certainly associated with virus infection.

Literature Cited

1. BRIERLEY, P. 1954. Symptoms in the florists' hydrangea caused by tomato ringspot virus and an unidentified sap-transmissible virus. Phytopathology 44: 696-699.
2. BRIERLEY, P., and P. LORENTZ. 1957. Hydrangea ringspot virus the probable cause of "running-out" of the florists' hydrangea. Phytopathology 47: 39-43.

DEPARTMENT OF PLANT PATHOLOGY, FLORIDA AGRICULTURAL EXPERIMENT STATION, GAINESVILLE, FLORIDA

CITRUS VARIETIES, SPECIES, AND RELATIVES SUSCEPTIBLE TO ATTACK AND DAMAGE BY THE BURROWING NEMATODE, RADOPHOLUS SIMILIS

W. A. Feder[1], H. W. Ford[2], J. Feldmesser[1], F. E. Gardner[1], R. F. Suit[2], A. Pieringer[2], and P. C. Hutchins[1]

Abstract

Nearly 400 varieties, species, and relatives of citrus, screened between 1953 and 1957, were found to be susceptible to damage by the burrowing nematode, Radopholus similis (Cobb) Thorne, the primary cause of spreading decline.

The burrowing nematode, Radopholus similis (Cobb) Thorne, an endoparasite, is the primary cause of the serious citrus disease known as spreading decline (4). All commercial citrus rootstocks used in Florida at present are susceptible to damage from this nematode. The fact that more than 1000 different kinds of citrus are available limits the use of field trials as a screening procedure until susceptible types have been eliminated.

A program designed to screen varieties, species, and relatives of citrus for resistance to attack by the burrowing nematode was started in 1953 by comparing the growth of seedlings in containers of infested and non-infested soil. The number of plants that could be screened each year was limited until more rapid procedures were developed in 1956. These techniques and methods are described in detail elsewhere (3).

Briefly the method consisted of planting candidate seedlings in soil tanks and greenhouse benches in which large numbers of burrowing nematodes were maintained by successive croppings to okra, a susceptible annual. The roots of the candidate seedlings were examined for visible lesions and the number of nematodes present was determined by incubation. Internal damage was detected by a rapid chromium trioxide staining technique. Seedlings of candidates showing less damage in the tests just described were studied by growing them in Petri dishes filled with sterile soil and inoculating their roots with known numbers of burrowing nematodes. The ability of the nematodes to penetrate the roots, lay eggs, and maintain populations was determined after 5 days by use of a modification of an aceto-osmium staining procedure and after 35 days by dissecting and counting the nematodes in the root systems.

The following species, varieties, hybrids or crosses, and relatives of citrus were susceptible to the burrowing nematode:

SWEET ORANGE:

Atlee	Donaldson	Magnum Morocco
Bedmar	Drake Star	Magnum Bonum
Bessie	Egyptian Blood	Majorca
Berna	Enterprise Seedless	Maltaise Sanguine
Brazilian	Florida Belle	Maltese Oval
Cadena Punchose	Futch Early	Mediterranean Blood
Cadenera Fina	Golden Ring	Mediterranean Sweet
Capuchin	Hamlin Cutting	McIlhenny
Centennial	Harvard # 2	Midget Honey
Chaffey's	Harvard Late	Non Pareil
Shamouti	Homosassa	Old Vini
Citrus Species	Honey Sweet	Orange de Nice
Cuba Sweet	Jaffa	Ovale Sangre
Du Fuya Mendu	Joppa	Paper Rind
Dugat	Lamb Summer	Parson Brown Carney
Du Roi	Laranja Gravo	Parson Brown Porter
Del Rio	Lue Gim Gong	Pelton
De Tetouan	Madam Vinous	Pineapple Cutting

[1]United States Department of Agriculture, Agricultural Research Service, Crops Research Division, Orlando, Florida.
[2]Florida Citrus Experiment Station, Lake Alfred.

SWEET ORANGE: (Continued).

Poorman Orange
Precocre de Valence
Princess Early Seedless
Rico #1
Rico #2
Rico #6
Ross Seedless
Ruby
Saint Michael
Sanford Mediterranean
Sangre Doble Fina
Seedless Late
Seedless Ruby
Selecta
Sweeguasse
Sweet Seedling
Taragrossa P.I. 103578
Taragrossa P.I. 105013
Valencia
Valencia Shambar
Viciedo P.I. 103579
Viciedo P.I. 105010
Zegzel
Zellwood

MANDARIN:

Batangus
Changsha
Chivka
Clementine
Choo Chou Tien Chieh
Cleopatra (Ponki)
Cleopatra Cutting
Cleopatra Seedling
Dancy Tangerine
Depressa
Haung Yen Mi Chieh
Honey Tangerine Nucellar
King of Siam
Kingerine Hybrid
Kino Kuni
Kunembo
Lau Chang
Mandarin
Maune Shui Chang
Miaray
Murcott Honey
Nicholson Early
Nobilis Avana
Nobilis
Oneco Tangerine
Scarlet Emperor
Sorrento Early
Sucrosa
Suen Kat
Summer
Sunki
Swatow
Swatow (Pongka Honey Orange)
Taad Chieh
Tankan
Taiwanica
Taiwanica P.I. 71238
Vermilian
Weshart Tangerine

MANDARIN (SATSUMAS):

Foley-Owari
Fugii Wase
Gyatokus Zairai
Hayashi #9
Iwakuma
Kama #3
Kawano
Lab Satsuma
Matsuda #17
Minata #16
Natsume Shin
Owari
Silverhill
Takano #29
Takazoe #25
Tako #8
Tanaka #22
Yakushiji
Yamazaki #10
Satsuma x Capuchin
Satsuma x Tangerine Seedling

GRAPEFRUIT (PUMMELO AND SHADDOCK):

Alamoen
Alsop
Avajan Shaddock
Blood Red (Canal Zone)
Burma Shaddock
Chin Kow Pummelo
China Cutting Pummelo
Chinese
Conner's Improved
Crimson
Cuban Shaddock C-10627
Davis
Dorsett's Wild
Duncan
India Red
Inman Late
Java
Leonardy
Little River
Misri Batabi
Mott
Mshikan
Nakon Pummelo
Navel Grapefruit
Ogami
Pink Pummelo
Pink Shaddock
Pumelo Aurantium
Red Mexican
Shambar
Siamese
Cuba Sweet
Tahiti Pink
Thong Dee
Tresca
Winston Early Seedless
Yu Tze Pummelo

LEMON:

Arizona Hardy
Batabi Shikan P.I. 137563-A
Batabi Shikan P.I. 137576-A
Bergametto
Bernia
Brazilian
Butnal Sweet
Citrus limon
Citrus limon Seedling
Columbia Sweet
Corregia
Davao
Des 4 Saisons
Eureka
Gombru
Harris
Harvey
Indian
Iran
Kadu Mul
Kusner
Limon Real #18
Limue Kahrgi P.I. 137567
Limue Kahrgi P.I. 136468
Lisbon C-12640
Lisbon C-12641
Meyer
Meyer Thornless
Morocco
Ponderosa
Punjab
Rough (Florida)
Rough (Lewis)
Simlac
Sp. P.I. 126539
Woglum
Variegated

LIME:

Balady	Kudzi Nebu	Rangpur
Eustis Limequat	Lakeland Limequat	Red Ling Mung
Kalpi	Ling Mung Seedling	Tavares Limequat
Key JRS	Nicaragua Mandarin Lime	Tavares Limequat Nucellar
Key L 487		

CITRON:

Citron	Etrog	Italian
Citron var. Yemen	Indian	Sicily

TANGELO:

Clemelo	Red	Tangelo C16-K-9
Clement	Satsumelo	Tangelo C18-S-8
County Farms	Sampson	Tangelo C-18-T-2
Mandelo	San Jacinto #4	Thornton
Minneola	Seminole	Umatilla
Minneola Nucellar	Seminole Nucellar	Watt
Nocatee	Summerall	Webber
Orlando	Sunrise	Wekiwa
Orlando Nucellar	Sunshine	Williams
Pearl Nucellar	Suwanee	Yalaha
Pina	Swingle	

SOUR ORANGE:

Algiers Seville	Chinotto	Sauvage Sour
Arancia Bizarria	Djeroek Bali	Seville
Bergamia	Kansu	Sour #2
Bergamotto	Natsume Dai Dai	Sour (Lab)
Bigardier	Natsu Mikan	Sour Variation
Bittersweet L-2-39	Ocklawaha Sour	Yuzu
Bittersweet L-2-40		

CITRANGE:

Cunningham Nucellar	Rusk	Savage
Morton	Sacaton Nucellar	Troyer
Phelps C-2717	Sanford	Willits
Phelps C-775		

CITRUS RELATIVES:

Aeglopsis chevalieri	Balsamocitrus paniculata	Microcitrus australasica
Afraegle gabonensis	Chalcas exotica	Poncirus trifoliata (Large Fl.)
Afraegle paniculata	Fortunella crassifolia	Poncirus trifoliata (Small Fl.)
Atalantia ceylanica	Fortunella japonica	Severinia buxifolia C-11667
Balsamocitrus daweii	Fortunella margarita	Severinia buxifolia C-11567

MISCELLANEOUS CITRUS:

Citrus amblycarpa	Citrus hystrix	Long Huang Kat
Citrus excelsa		

MISCELLANEOUS HYBRIDS:

Balang	Citrumelo C 4481	Merritt Island #7
Calamondin	Davis x Dancy	Nippon Kumquat
Calamondin x Grft.	Glen Citrangedin	Norris x Trifoliata
Citrangedin	Jaffa x Trifoliata	Minneola x Valencia
Citrangequat L 48029	Japansche Citroen	Orangeuma
Citrangequat C 48032	Kara Seedling	Pineapple x Trifoliata
Citrangequat Telfair	Limequat x King	Pommosat
Citrangor C 42681	Lemonquat	Rangpur x Sour Pummelo
Citrangor C 43728	Leonardy x Trifoliata	Sinton Citrangequat
Citremon	Merritt Island #5	Tangelo x Sweet Orange

MISCELLANEOUS HYBRIDS: (Continued).

Tangor (Ry x Tn) C 539	Temple x Trif.	Trifoliata x RL-A
Tangor (Med. Sw. x Tn)	Thesnol	Umatilla x Valencia
Tangor (Ry x Tn) C 49661	Tresca x Dancy	Wilking Seedling

No material tested so far has been found immune.
The following selections showed some tolerance (1, 2):

Sweet Orange: Pineapple, Sanguina grosse ronde
Shaddock: Cuban
Lemon: Ichang, Ichangensis
Citron: Sand Field
Tangelo: C 18-E-7, C 16-J-15, C 18-W-5
Citrange: Carrizo
Citrus relative: Microcitrus australis
Miscellaneous hybrids: Thomasville citrangequat, Lemelo

All seed used so far in the screening program was obtained from the United States Department of Agriculture Experimental Farm, Orlando, Florida; the Citrus Experiment Station, Lake Alfred, Florida; and the Royal Purple Nurseries, Orlando, Florida.

Literature Cited

1. FEDER, W. A., and H. W. FORD. 1957. Susceptibility of certain citrus varieties, species, and relatives to the burrowing nematode. Proc. Florida State Hort. Soc. 70: 60-63.
2. FORD, H. W. 1956. Citrus rootstock selections tolerant to the burrowing nematode. Proc. Florida State Hort. Soc. 69: 44-51.
3. FORD, H. W., and W. A. FEDER. 1958. Procedures used for rapid evaluation of citrus for resistance to certain endoparasitic nematodes. Proc. Amer. Soc. Hort. Sci. 71: In press.
4. SUIT, R. F., and E. P. DuCHARME. 1953. The burrowing nematode and other parasitic nematodes in relation to spreading decline of citrus. Plant Disease Reptr. 37: 397-383.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE; AND FLORIDA CITRUS EXPERIMENT STATION

NEMATODES ASSOCIATED WITH ROOT INJURY OF MUSA SPP. IN HONDURAN BANANA SOILS

R. H. Stover and Max J. Fielding[1]

Abstract

Twelve species of plant parasitic nematodes were obtained from banana soils and Musa spp. roots in Honduras. Four of these, Meloidogyne arenaria, Hoplolaimus sp., Radopholus similis, and Pratylenchus musicola, were encountered consistently and in abundance. Root injury was more prevalent in sandy loam than in loam or clay loam soils. There was no visual evidence of abnormal growth above ground.

Bananas (Musa acuminata) are intensively cultivated in many tropical areas. Until recently there were only two reports of the edible or non-cooking varieties being afflicted with disease or poor growth associated with root injury caused by nematodes. Cobb (1) attributed a disease of bananas in Fiji to Radopholus similis (Cobb, 1893) Thorne, 1949, and found the same species associated with a disease of the Gros Michel banana in Jamaica called "black head" (2). In Grenada, Cobb (3) found Pratylenchus musicola (Cobb, 1919) Filipjev, 1936 associated with a disease of the bluggoe banana or plantain, a cooking variety (M. x paradisiaca). Other parasitic genera of nematodes have been obtained from Musa spp., but these have not been associated with any specific diseased condition or abnormal growth (Table 1). Three species of nematodes have been found associated with root injury in abaca (M. textilis) (14).

In our studies on factors influencing the rate of spread of banana wilt caused by Fusarium oxysporum f. cubense in different soils, the possible role of nematodes is being investigated because of their known importance in other wilt diseases (10). As part of these investigations, a survey was made to determine the most prevalent nematodes in banana growing areas in Honduras. The results of this survey are reported in this paper.

METHODS

From 15 to 20 banana (M. acuminata), abaca (M. textilis), or plantain (M. x paradisiaca) roots 4 to 8 inches long (1/8 to 1/4 inch diam.) were taken at random around the pseudostem or mat during May and June, 1955. In the laboratory the adhering soil was shaken and brushed off and a measured volume screened for nematodes, using a modification of the Büchner method previously described by Feder and Feldmesser (6).

The roots were washed and those showing lesions were cut into 1/4 to 1/2 inch segments. These were thoroughly mixed and a composite sample of 10 or 20 g was placed in a Waring blendor for 15 seconds with sufficient water for blending. Nematodes were extracted from this blended material after the method of Taylor and Loegering (14).

Samples were taken from three general areas in Honduras. The largest area consisted of alkaline clay loam and loam soils near La Lima currently producing Gros Michel bananas, and an acid sandy loam area at Lancetilla and Guaymas on which most of the bananas had been destroyed by banana wilt 25 years ago. Plantain and abaca roots sampled were growing in the Guaymas area. One collection of "Chato" plantain roots was made in a sandy loam area at Choloma where Gros Michel bananas were destroyed by wilt 30 years ago.

RESULTS

In Table 2 are listed the 12 species of plant parasitic nematodes found in banana soil and Musa spp. roots. Only four of the 12 species were encountered consistently and in abundance. These were Meloidogyne arenaria, Chitwood, 1949, Hoplolaimus sp., Radopholus similis, and Pratylenchus musicola. Sampling was not great enough to determine definite differences in the nematodes found in the different soils. There was an indication, however, that R. similis

[1]Formerly at the Louisiana Agricultural Experiment Station; now with the E. I. Du Pont Company.

Table 1. Plant parasitic nematodes found in association with Musa spp. in Honduras.

Genus	Species	Musa Hosts	Reported by[a]
Pratylenchus	P. musicola (Cobb) Filipjev	Banana, abaca plantain	Cobb(3), Goodey(8), Taylor & Loegering(14), This report.
Radopholus	Unidentified	Banana	Sher(12)
	R. similis (Cobb) Thorne	Banana, abaca, plantain	Cobb(2), Taylor & Loegering(14) This report.
Meloidogyne	Meloidogyne	Banana, abaca	Cobb(1), Fahmy(5), Ocfemia & Calinisan(13)
	M. incognita var. acrita Chitwood	Abaca	Taylor & Loegering(14)
	M. arenaria	Banana	This report.
Rotylenchulus	R. multicinctus	Banana	Goodey(9), Filipjev(7)
	Unidentified	Banana	This report.
Xiphinema	X. americanum	Banana	This report.
	X. ensiculiferum (Cobb) Thorne	Banana	Cobb(2)
Hoplolaimus	Unidentified	Banana	Sher(12)
	N. sp.	Banana	This report.
Helicotylenchus	H. nanus	Banana	This report.
	N. sp.	Banana	This report.
Paratylenchus	Unidentified	Banana	This report.
Tylenchus	T. davainii Bastian	Banana	Filipjev(6), Crossman & Christie(4)
	T. filiformis Bütschli	Banana	Filipjev(6, pg. 264 (?)) This report.
Tylenchorynchus	Unidentified	Banana	This report.
	T. claytoni Steiner	Banana	This report.
Aphelenchoides	A. parietinus (Bastian) Steiner	Banana	Rahm(11)

[a]See references.

Table 2. Nematode species encountered in banana soils and in Musa spp. roots from Honduras.

	Loam - clay loam				Sandy loam	
	Banana roots		Soil		Abaca, plantain roots	
No. samples examined --	10		44		12	
Species	Times[a] encountered	Nos.[a] found	Times encountered	Nos. found	Times encountered	Nos. found
Meloidogyne arenaria[b]	6	+++	40	++++	2	++
Helicotylenchus sp.	0	0	8	++	4	+
H. nanus	0	0	1	+	0	0
Hoplolaimus sp.	6	+++	15	+++	1	+
Paratylenchus sp.	0	0	2	+	0	0
Xiphenema americanum	0	0	1	+	0	0
Tylenchorhynchus sp.	0	0	1	+	0	0
T. claytoni	0	0	1	+	0	0
Tylenchus filiformis	0	0	1	+	0	0
Pratylenchus musicola	1	++	0	0	8	++++
Radopholus similis	0	0	1	++	9	+++++
Rotylenchus sp. larvae	0	0	1	++	0	0

[a]+++++, very numerous; ++++, numerous; +++, fairly numerous; ++, commonly encountered; +, infrequently encountered.

[b]Meloidogyne larvae encountered in soil considered to be M. arenaria as all galls encountered on banana roots containing mature females were M. arenaria.

and P. musicola were most prevalent in abaca and in roots from three varieties of plantain from the sandy loam areas Only an occasional plant of Gros Michel had survived the ravages of Fusarium wilt in the sandy loam areas and the limited sampling of these yielded P. musicola and R. similis in only one sample. In numerous samplings of Gros Michel banana roots and soil from the clay loam area, P. musicola and R. similis were only encountered once. R. similis was prevalent in the "Chato" plantain roots from Choloma. The nematodes found in plantains and abaca growing on sandy loam soils consisted of R. similis and P. musicola in abundance; M. arenaria was commonly encountered and occasionally Helicotylenchus spp. Root lesions were much more prevalent on all Musa spp. growing in sandy loam soils than on those in loam and clay loam soils. The only parasitic nematodes found in abundance in loam and clay loam soils, the areas where bananas are now produced, were Hoplolaimus sp. and Meloidogyne arenaria.

Root injury associated with the presence of plant parasitic nematodes consisted of destruction of root tips, resulting in root proliferation above the damaged area, and brown to black elongated lesions above the growing points. Galling was also common on small roots in areas where Meloidogyne sp. was present. In two samples Hoplolaimus sp. was found in large numbers inside the lesions on banana roots.

Literature Cited

1. COBB, N. A. 1893. Nematodes, mostly Australian and Fijian. MacLeay Memorial Volume, Linnean Society of New South Wales. pp. 252-308.
2. COBB, N. A. 1915. Tylenchus similis, the cause of a root disease of sugar cane and banana. J. Agric. Res. 4: 561-568.
3. COBB, N. A. 1919. A new nema, Tylenchus musicola, n. sp., said to cause a serious affection of the bluggoe banana in Grenada, British West Indies. West Indian Bulletin 17: 179-182.
4. CROSSMAN, L., and J. R. CHRISTIE. 1937. Lists of plants attacked by miscellaneous plant-infesting nematodes. Plant Disease Reptr. 21: 144-167.
5. FAHMY, T. 1924. A banana disease caused by a species of Heterodera. Egypt, Ministry of Agriculture, Technical and Scientific Service Bulletin No. 30. 11 pp.
6. FEDER, WILLIAM A., and JULIUS FELDMESSER. 1954. The Büchner funnel as an aid in collecting and concentrating nematode populations. Plant Disease Reptr. 38: 805-806.
7. FILIPJEV, I. N., and J. H. SHUURMANS STEKHOVEN. 1941. A manual of agricultural Helminthology. E. J. Brille, Leiden.
8. GOODEY, T. 1928. Observations on Tylenchus musicola Cobb, 1919, from diseased banana roots. J. Helminthology 6: 193-198.
9. GOODEY, T. 1951. Soil and fresh water nematodes. John Wiley and Sons, Inc. New York.
10. HOLDEMAN, Q. L. 1954. Nematodes as possible members of disease complexes involving other plant pathogens. Plant Disease Reptr., Suppl. 227: 77-79.
11. RAHM, GILBERT. 1929. Nematodas parasitas o semi-parasitas de diversas plantas culturaes do Brasil. Arch. Inst. Biol. São Paulo 2: 67-136.
12. SHER, S. A. 1954. Observations on plant-parasitic nematodes in Hawaii. Plant Disease Reptr. 38: 687-689.
13. OCFEMIA, G. O., and M. R. CALINISAN. 1928. The root-knot of abaca or Manila hemp. Phytopathology 18: 861-867.
14. TAYLOR, A. L., and W. Q. LOEGERING. 1953. Nematodes associated with root lesions in abaca. Turrialba 3: 8-13.

DEPARTMENT OF PLANT PATHOLOGY, VINING C. DUNLAP LABORATORIES, TELA RAILROAD CO., LA LIMA, HONDURAS. (A SUBSIDIARY OF UNITED FRUIT CO.)

REPRODUCTION AND SYMPTOMATOLOGY OF CERTAIN ECTOPARASITIC NEMATODES ON ROOTS OF THOMPSON SEEDLESS GRAPE

D. J. Raski and J. D. Radewald[1]

Summary

Seven replications of 100 hand-picked specimens each of Xiphinema index, Criconemoides xenoplax, Paratylenchus hamatus, and Trichodorus christiei from various vineyard soils in California were tested on Thompson seedless rootings in sterile soil. T. christiei did not survive; the other nematodes multiplied, and visual observation of specimens feeding on the roots confirmed the parasitism of all three species. Obvious root injury was observed only in the case of X. index, where feeder roots were destroyed or malformed by root bending, swelling, and cortical necrosis.

Nematode infestations of California vineyards are characterized by the diversity of the plant-parasitic species in and about the vine roots (1, 2). Among the species are many ectoparasitic types whose role in grape production has not been determined. To study the parasitism on grapevines of four of these species, experiments were begun on April 19, 1957, with the following populations: 1) Xiphinema index Thorne & Allen, 1950, from Vineyard A, Tokay var., Lodi; 2) Criconemoides xenoplax Raski, 1952, from Vineyard B, Tokay var., Lodi; 3) Paratylenchus hamatus Thorne & Allen, 1950, from Vineyard C, Tokay var., Lodi; 4) Trichodorus christiei Allen, 1957, from Vineyard D, Thompson seedless var., Kingsburg.

Infested soil from each field was wet-screened, and individual nematodes were hand-picked into clean tap water to separate them from other nematode species present in the washings. One-year-old two-bud Thompson seedless vines rooted in sterile soil were used for the test. The roots of the plants were washed free of soil, the tops and roots were pruned back, and fresh weights were obtained before the plants were transplanted into sterile soil in 6-inch clay pots. Seven vines were each infested with 100 specimens of each nematode species by pouring water containing the nematodes into four shallow holes made near the crown of each plant. After the water had been absorbed the holes were closed by scratching the surface of the soil and immediately irrigating each pot.

The plants were grown under greenhouse conditions for 142 to 147 days, after which the soil was carefully separated from the roots. Nematode populations in each pot were determined by washing the roots vigorously and wet-screening the washings. The soil was measured by volume and a 250 cc aliquot portion was wet-screened. A series of 20-, 100-, and 200-mesh screens were used for wet-screening the root wash water and the soil, and the 20-mesh screening was discarded in each case. The only exception was the series infested with P. hamatus, in which a 325-mesh screen was substituted for the 200-mesh screen. Counts of aliquots of each screening were made on 1-cc counting slides, and the figures shown in Table 1 include the sum of counts from both soil and root washings. Fresh weights were obtained from the plants after the roots had been washed, and the increase in weight of each plant was recorded.

The failure of T. christiei to survive indicates that grape may not be a suitable host for this parasite. However, with different temperature and/or moisture conditions of the soil, feeding and reproduction might have taken place. Further tests are planned to determine what conditions might favor development of this nematode on the roots of grape.

The other nematodes tested reproduced in great numbers, and visual observations of specimens actually feeding on the roots confirmed the parasitism of all three species The effects on roots produced by C. xenoplax and P. hamatus were not particularly notable, although the latter produced shallow, localized lesions. Further tests are in progress to determine more exactly what effects these nematodes produce on grape roots, including any histological changes.

---

[1]Respectively, Associate Nematologist and Research Assistant, Department of Plant Nematology, University of California, Davis.

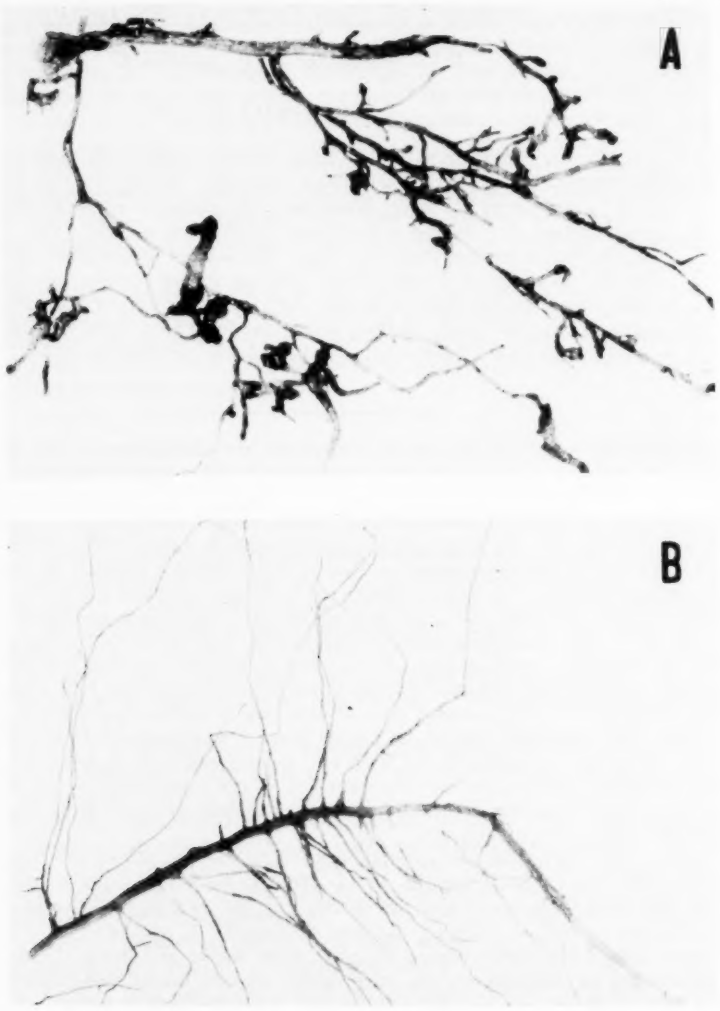


FIGURE 1. Roots of Thompson seedless grapevines grown in:
A) Sterilized soil infested with Xiphinema index.
B) Sterilized soil.

Table 1. Nematode populations developed from 100 specimens after 5 months on roots of Thompson seedless grapevines.

Replicate	Check	Criconemoides xenoplax	Xiphinema index	Paratylenchus hamatus	Trichodorus christiei
1	0	6,942	48,800	15,475	0
2	0	6,380	50,528	94,979	0
3	0	2,830	52,640	22,840	0
4	0	6,312	35,170	119,904	0
5	0	6,152	51,660	19,068	0
6	0	4,040	38,392	35,140	0
7	0	10,280	22,697	14,560	0
Average	0	6,134	42,841	45,995	0

By contrast the disease symptoms associated with parasitism by X. index were very pronounced, including extreme malformation and reduction of feeder rootlets. The growing points of many laterals were destroyed, which prevented further growth of those rootlets. Other symptoms included root bending, swelling, and extensive cortical necrosis (Fig. 1, A, B). In general the symptomatology was similar to that associated with parasitism by Xiphinema diversicaudatum of rose, peanuts, fig, and other plants, as described by Schindler (3).

The increases in fresh weights during the period of this test showed slight differences between infested and uninfested plants, but these differences were not statistically significant. Longer-range tests are planned to determine whether continued parasitism would eventually reduce plant growth. The extreme root injury resulting from parasitism by X. index, however, makes it highly probable that this species is a serious parasite that causes reductions in vine growth and production in many parts of California. This widely distributed species has been found in vineyards in Santa Clara, Napa, and Sonoma Counties, of the coastal regions, and in San Joaquin, Merced, Fresno, Kings, and Tulare Counties, of the San Joaquin Valley.

Literature Cited

1. RASKI, D. J. 1954. Soil fumigation for the control of nematodes on grape replants. Plant Disease Reptr. 38: 811-817.
2. RASKI, D. J. 1955. Additional observations on the nematodes attacking grapevines and their control. Am. Jour. Enology 6: 29-31.
3. SCHINDLER, A. F. 1957. Parasitism and pathogenicity of Xiphinema diversicaudatum, an ectoparasitic nematode. Nematologica 2: 25-31.

DEPARTMENT OF PLANT NEMATOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS

CONTROL OF THE COTTON ROOT-KNOT NEMATODE ON EXTRA-LONG-STAPLE COTTON[1]

Harold W. Reynolds[2]

Abstract

The economic importance and methods of control of the cotton root-knot nematode on extra-long-staple cotton are discussed, and the results of a field-scale fumigation experiment are reported. A highly significant increase in yield of cotton was secured with pre-planting soil fumigation. Effective control measures practiced in the Southwest are crop rotation, summer fallow and soil fumigation.

Essentially all the extra-long-staple cotton, Gossypium barbadense L., produced in the United States is grown in Arizona, New Mexico, and west Texas. This crop has become very important in southwestern agriculture in recent years. In 1956 there were 41,000 acres of Pima S1 extra-long-staple cotton while in the following year 82,000 acres were grown in the area. This increase in acreage of extra-long-staple cotton in recent years was brought about by several factors, among which are 1) the introduction of a new high-yielding variety, Pima S1, 2) the suitability of many southwestern irrigated soils and climatic conditions to the production of high-quality lint, and 3) the development of a satisfactory market for high-quality extra-long-staple fiber. Unfortunately the extra-long-staple cottons are more susceptible to the cotton root-knot nematode, Meloidogyne incognita acrita Chitwood, than the short-staple varieties, G. hirsutum L.

The economic importance of the cotton root-knot nematode on extra-long-staple cotton in Arizona has been recognized for several years. Mr. C. J. King, Superintendent of the U. S. Field Station, Sacaton, Arizona, stated in correspondence in 1933: "The discovery that nematodes are an important problem in the commercial production of Pima cotton in parts of the Salt River Valley has prompted us to consider the possible influence of these organisms on our field station experiments, and on cotton work in general in areas where they are prevalent. It seems apparent that the disease [root-knot] was affecting our experiments at Sacaton and possibly influencing our opinions regarding varieties and cultural treatments as far back as 1920."

Even light root-knot nematode populations in sandy soils cause economic loss in this crop. Most growers prefer sandy loams for growing Pima S1, to prevent undesirable rank growth, which occurs on most fine-textured soils. As a result, much land which once contained only very light root-knot nematode populations, because of control through crop rotation, is being continued in cotton year after year. This system of cropping, along with an increase in acreage of extra-long-staple cotton, requires that more attention be given to control of the root-knot nematode.

Symptoms: The direct effect of the root-knot nematode is the formation of knots or galls on the roots. Heavily knotted roots are abnormally short and have fewer lateral "feeder" roots than normal root systems. Since the tissues of the knotted roots are disarranged, these roots are inefficient in conducting water and nutrients. Being short and lacking in lateral roots, they do not reach as large a volume of soil as normal roots. Consequently the plant often cannot obtain sufficient water and nutrients from the soil and appears to be under stress, often wilting during the heat of the day. Root-knot nematodes by themselves stunt but never kill cotton plants, but combinations of root-knot nematodes and certain fungi may kill seedlings (1).

Distribution and Spread of the Nematode: The eggs and larvae of the cotton root-knot nematode are spread through the field by irrigation water, infested crop residues and soil adhering to farm machinery. Also waste water from a field carries the parasite from field to field and often from one farming area to another. In fact, an examination of soil in field head ditches and larger lateral ditches showed the presence of several plant parasitic nematodes including the cotton root-knot nematode.

---

[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Arizona Agricultural Experiment Station.

[2]Nematologist, Agricultural Research Service, Cotton Research Center, Tempe, Arizona.

General Control Measures: There are three effective and practical means of controlling the cotton root-knot nematode: crop rotation, summer fallow, and soil fumigation. Rotation of cotton with a variety of alfalfa resistant to the cotton root-knot nematode, such as African or Chilean, for 2 or more years has proved to be a good method of reducing root-knot populations on the following cotton crop. In addition, this rotation has the advantage of maintaining soil fertility at a high enough level for good cotton yield. A 1-year rotation of small grain crops grown during the cool months, followed by grain sorghum in the summer, has also reduced root-knot nematode damage to the cotton crop the next year.

Root-knot nematodes are controlled effectively if the land is kept completely fallow during the hot summer months with an occasional discing of the soil. This eliminates weeds on which the nematodes can feed, and keeps the upper 8 or 9 inches of the soil dry and unfavorable for nematode survival. This type of control, by drying and starvation, is very rapid and effective under arid southwestern conditions. However, some growers cannot justify fallowing their land because of limited acreage or other factors which might disrupt their plan of operation.

Where it is not possible for growers to rotate crops or to practice summer fallow for root-knot control, soil fumigation has given very good results on fields planted to cotton year after year. Accordingly a large-scale field experiment was established in 1956 to determine the advisability of soil fumigation where extra-long-staple cotton was grown.[3]

Large-scale Fumigation Test: The field in which this experiment was established is located at Queen Creek, Arizona, on the ranch of Barney and Meecham. Pima S1 cotton was planted in the field in both 1954 and 1955, and root-knot nematodes were troublesome both years.

The field, fumigated on March 23, 1956 when soil moisture was optimum, consisted of listed beds relatively free of cotton stubble and roots of the previous crop. The experiment consisted of eight treatments randomized in blocks. Each treatment was replicated four times. Each plot was 0.36 acre (four beds 38 inches wide and 1230 feet long). The entire test totaled approximately 11.5 acres. The soil fumigants[4] used, including active ingredients and rates of application per acre, are shown in Table 1.

The first six treatments were applied with four-row, eight-chisel injection equipment on a row-placement basis, in two lines 6 inches from the center of the listed bed. The fumigants were injected approximately 8 inches below the normal surface level. The soil was compacted or a soil surface seal was made by towing a ring roller 12 feet wide behind the injection equipment. The cotton was planted March 31. The post-planting treatment, with granular Nemagon, was applied as a side dressing with a fertilizer drill on May 31. The granules were placed 6 inches deep and 12 inches from the cotton row on both sides.

On November 2 and 3 the plots were harvested with two mechanical pickers. All four rows of each plot were harvested, each machine picking two rows in each plot. Seed-cotton weights were determined by weighing the load at a nearby gin immediately after each plot of each replicate was harvested. The second and final picking was made on November 20 and 21.

As soon as the cotton was harvested, randomly selected plants from each plot were examined for incidence of root-knot nematodes. Each plant was given a relative root-knot index rating as described by Smith and Taylor (2) and these data are recorded in Table 1.

Results: Four weeks after seedling emergence, differences were noted in size of plants on fumigated and unfumigated plots; somewhat larger and more vigorous seedlings occurred on the fumigated plots. Ten weeks after seedling emergence these differences in both size and uniformity were considerably greater, as shown in Figure 1.

All fumigants applied as preplanting treatments gave highly significant increase in yield over postplanting treatments and no treatment. Nematox 45 at the rate of 4 gallons per acre gave a highly significant increase in yield over the other fumigants regardless of rate of application. However, there was not a significant increase in yield with Nematox at 4 gallons over 3 gallons per acre.

---

[3]Grateful acknowledgment for assistance in establishing the experiment and determining yields is expressed to Stuart Evans of Arizona Fertilizers, Inc. Acknowledgment is also expressed to the Eston Chemical Co. and Shell Chemical Corp. for furnishing nematocides for the experiment.

[4]To simplify presentation trade names of nematocides are used. No endorsement of these products and no criticism of similar products not tested are intended.

Table 1 Effect of fumigants on yield and root-knot index of Pima S1, an extra-long-staple cotton, Queen Creek, Arizona, 1956

Fumigant and rate per acre	Active ingredient per acre (pounds)	Yield of lint per acre[a] (pounds)	Relative root-knot index[b]
Bromofume 85:[c]			
3 gallons	36	901	2.3
2 1/4 gallons	27	950	2.3
D-D:[d]			
12 gallons	120	967	2.2
9 gallons	90	946	2.6
Nematox 45:[e]			
4 gallons	17.3	1041	0.8
3 gallons	13	1013	1.2
Nemagon-impregnated granules of Attaclay:[f]			
100 pounds	10	596	3.5
None (Control)		514	3.7
L.S.D. 0.05		51	0.4
0.01		69	0.5

[a]Factor for converting seed cotton to lint was 32 percent; mean for 4 replications.
[b]0, no infection; 1, trace; 2, light; 3, moderate; 4, heavy; mean for 4 replications.
[c]Trademarked material furnished by Eston Chemical Co. and containing 83 percent by weight of 1, 2-dibromoethane.
[d]Furnished by Shell Chemical Corp., containing 50 percent by weight of 1, 3-dichloropropene; the remainder mostly 1, 2-dichloropropane.
[e]Furnished by Arizona Fertilizers, Inc., containing 45.4 percent by weight of "Nemagon", a product of Shell Chemical Corp. Active ingredient of "Nemagon" is 1, 2-dibromo-3-chloropropane.
[f]Attaclay, an inert product of the Attapulgus Clay Co., is a complex hydrated aluminum magnesium silicate.

FIGURE 1. Pima S1 cotton growing on fumigated and unfumigated soil. The plot on the left received a preplanting treatment of Nematox 45 at 4 gallons per acre. Center plot was not treated. The plot on the right received Nematox 45 at 3 gallons per acre. These rates are equivalent to active ingredient "Nemagon" at 1 gallon and 3/4 gallon per acre respectively.

The postplanting treatment (Attaclay granules impregnated with Nemagon) gave somewhat better yield than untreated plots. No difference was noted in the rate of growth in the postplanting treatment for 6 weeks. At that time the plants started showing better growth and a heavier boll set than the plants on the untreated plots. By late October a still heavier set of immature bolls was observed but the plants were severely damaged by an early frost. This damage probably accounts for the relative lack of effective control.

This experiment demonstrates that growers should give careful attention to checking fields for cotton root-knot nematodes. If the fields are affected by root-knot and are to be planted to extra-long-staple cotton, the pest should be controlled through crop rotation, summer fallow, or soil fumigation.

Literature Cited

1. REYNOLDS, H. W., and R. G. HANSON. 1957. Rhizoctonia disease of cotton in presence or absence of the cotton root-knot nematode in Arizona. Phytopathology 47: 256-261.
2. SMITH, A. L., and A. L. TAYLOR. 1947. Field methods of testing for root-knot infestation. Phytopathology 37: 85-93.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, AND ARIZONA AGRICULTURAL EXPERIMENT STATION

SOURCES OF RESISTANCE TO RACES 6 AND 13A OF THE OAT STEM RUST FUNGUS[1]

J. Artie Browning[2]

Summary

Eight strains from the World Collection of Oats appear to be new sources of resistance to the common oat stem rust races 7, 7A, and 8, and also to the rare but potentially important races 6 and 13A. The combined resistance of these oat strains to these five races indicates they may contain one or more new genes for stem rust resistance.

Race 6 of the oat stem rust fungus was first described from a collection made in 1925 in Saskatchewan as being able to attack all known sources of stem rust resistance (3, 4). It was collected as a rare race from the Prairie Provinces of Canada for several years (7) but it has never become established in the annual north-south rust-movement of Central North America. Race 6 has, however, been collected regularly from the barberry areas of northeastern United States since 1950 (9) and poses a possible threat to oat production there and throughout the oat growing regions of North America.

The isolation of race 6 from an aecial collection from barberry in Illinois in 1954 (9) and from oats in separate regions of Missouri in 1955 (10) and 1956 (11) indicates that race 6 is now present in the Mississippi Valley.

The only genetic resistance to race 6 so far reported in a hexaploid oat is from the variety Hajira (C.I. 1001) (12, 13). The gene conditioning this so-called (6) "Canadian" type of resistance confers a high degree of resistance at cool or moderate temperatures (Fig. 1) but loses its effectiveness when the host plant is grown at temperatures above 75° to 80° F. (12). Fortunately, temperatures are seldom high enough for a sufficient period of time to cause this breakdown in the field. That breakdown of the "Canadian" resistance can occur in the field, however, was observed in Minnesota in 1948 (6).

As race 6 is a serious threat to oat production in the Mississippi Valley, and in view of the instability of the "Canadian" gene which conditions resistance to this race, the World Collection of Oats was screened for other sources of resistance. The results of this investigation are reported herein.

MATERIALS AND METHODS

Approximately 4700 strains from the World Collection of Oats[3] were tested in the seedling stage in the greenhouse during the winter of 1954-55 for reaction to race 6 of the oat stem rust fungus (Puccinia graminis Pers. f. sp. avenae Erikss. & Henn.). Each entry was planted in a clump (about 10 seeds per clump) with a 2-inch spacing between clumps in benches filled with sand. The oat seedlings were inoculated when about 4 inches tall with inoculum increased from a single pustule isolate of race 6, by dusting the leaves with a talc suspension of spores using a cyclone spore separator.

Leaves were moistened before and after inoculation with a 1 percent solution of Tween-20. The inoculated plants were kept overnight under a wire frame covered with wet burlap and plastic sheeting. Infection types were assigned each entry according to the method of Bailey (2) about 12 days following inoculation. Entries which showed any degree of resistance were retested. In other tests those strains which showed a resistant reaction were tested with a

---

[1]Cooperative investigations between Iowa State College and Crops Research Division, Agricultural Research Service, United States Department of Agriculture. Journal paper No. J-3460 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1176.

[2]Associate Professor of Plant Pathology, Iowa State College.

[3]Seed was supplied by David J. Ward from the World Collections of Small Grains of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

mixture of isolates of race 6[4], and with races 7, 7A, 8, and 13A[5].

RESULTS

A total of 147 strains from the World Collection of Oats showed resistance to race 6[6]. Lines which were resistant to the purified inoculum were also resistant to the mixture of isolates of race 6. Since the resistance of most strains could be traced to the known "Canadian" resistance they were not considered further. Resistant entries not known to have Hajira in their parentage are listed in Table 1. Of these, the entries with higher C. I. Nos. likely have a mistaken designation of parentage or are the result of seed admixture or of outcrossing with a line containing the "Canadian" resistance gene. Eight strains that seem to be most promising as possible new sources of resistance are C.I. 2413 and 2710, unnamed entries from China; C.I. 3030 and 3031, unnamed hull-less entries from Africa; and C.I. 3037, 3038, 3039, and 3040, all selections out of variety Kherson from Africa. The reactions of these entries and of the varieties Richland (C.I. 787), Canuck: Hajira x Jostrain (C.I. 4024), and White Tartar (C.I. 551), to races 6, 7, 7A, 8, and 13A of the oat stem rust fungus are given in Table 2.

Table 1. Strains from the World Collection of Oats which were resistant to race 6 of the oat stem rust fungus and which are not known to have Hajira in their parentages.

C.I. No.	Name or Parentage	Source	Reaction
2382	Nortex	Texas	Segregating
2413	Unnamed	China	Resistant
2710	Unnamed	China	Resistant
3030	Hull-less	Africa	Resistant
3031	Hull-less	Africa	Segregating
3037	Kherson No. 27	Africa	Resistant
3038	Kherson No. 99	Africa	Resistant
3039	Kherson No. 22	Africa	Resistant
3040	Kherson No. 34	Africa	Resistant
4159	Columbia x (Victoria x Richland)	Canada	Segregating
4318	Victoria x Norton	Georgia	Segregating
5875	Fulwin x (Lee-Victoria) x Bonda	Maryland	Segregating
6576	Atlantic x (Clinton[2]-Santa Fe)	Idaho	Resistant
6766	Clintafe Purification	Iowa	Resistant
6858	Probably Avena strigosa	Turkey	Resistant

Table 2. Average range of infection types of certain oat strains to key races of Puccinia graminis avenae.

Oat Strain	Race 6	7	7A	8	13A
Richland (C.I. 787)	4	1	1	4	4
Canuck (C.I. 4024)	1	1	4	1	4
White Tartar (C.I. 551)	4	4	4	2	4
C.I. 2413	1^{+}-3^{-}	2	2^{+}-3^{-}	1^{+}-2^{-}	2^{-}
C.I. 2710	1-X^{-}	2-X^{-}	X^{-}-3	1^{+}-2^{-}	1-2^{-}
C.I. 3030	1-2	2-2^{+}	2^{+}-3	1^{+}-2^{-}	1-2^{-}
C.I. 3031	1-X^{-}	2^{+}-3	3	1^{+}-2^{-}	2-X^{-}
C.I. 3037	1-2^{+}	2-3^{-}	2-X^{-}	1^{+}-2^{-}	1-2
C.I. 3038	1-X^{-}	2^{+}-3^{-}	X-3	1^{+}-2^{-}	2-X^{-}
C.I. 3039	1-2^{+}	2^{+}-3^{-}	2^{+}-X^{-}	1^{+}-2^{-}	2^{-}
C.I. 3040	1-2^{+}	2^{+}-3	1^{+}-X^{-}	1^{+}-2^{-}	2^{-}

[4]The mixture of isolates of race 6 was supplied by Dr. E. C. Stakman, Federal Rust Laboratory, St. Paul 1, Minnesota.

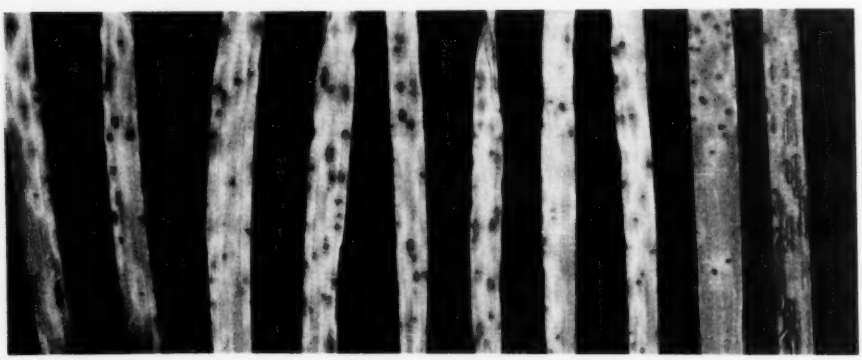
[5]Inoculum of race 13A was supplied by Dr. J. J. Christensen, Federal Rust Laboratory, St. Paul 1, Minnesota.

[6]The reactions of all entries in the World Collection of Oats can be obtained from David J. Ward, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

The reactions to race 6 of oat strains containing the known stem rust resistance genes in different combinations are shown in Figure 1. The reactions of the possible new sources of resistance to race 6 are shown in Figure 2. The eight new sources of resistance do not offer resistance of as high a degree as does Richland to races 7 and 7A, or Canuck to races 6, 7, and 8. However, the new sources are the only ones known to have combined resistance to races 6, 7, 7A, 8, and 13A.

Abcd abcD aBCd AbcD aBCD ABCd ABCD 7-0-3 abcd
Race 6

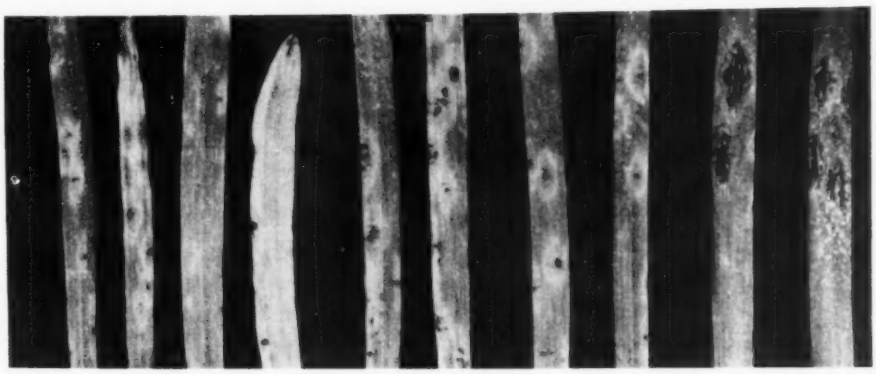
FIGURE 1. Seedling reactions to stem rust race 6 of oat varieties containing known stem rust resistance genes in different combinations. "A" designates the Richland gene for oat stem rust resistance (13), "BC" the "Canadian" gene (13), and "D" the White Tartar gene (8). From left to right, C.I. 787 (Richland), 551 (White Tartar), 4024 (Canuck), 7144, 6537 (Burnett), 6662 (Garry), 7145, 7-0-3 (Sevnothree, 3251, similar in rust reaction to Jostrain), and C.I. 2053 (Markton).



2413 2710 3030 3031 3037 3038 3039 3040 aBCd abcd
Race 6

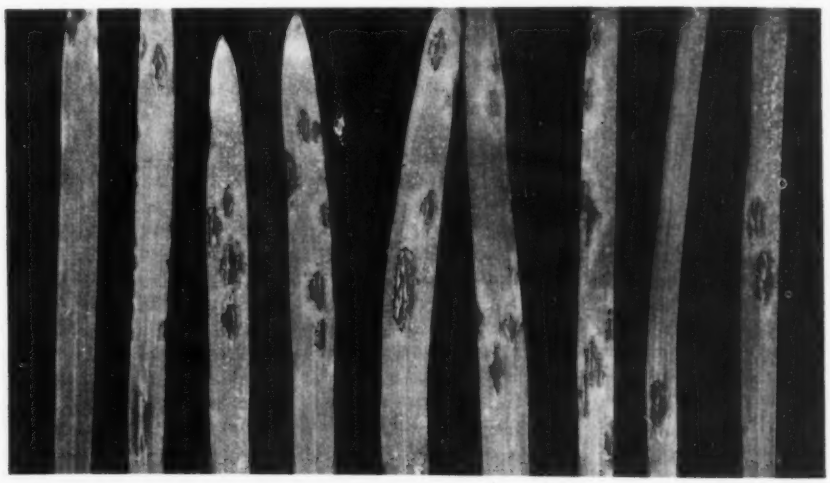
FIGURE 2. Seedling reactions of eight C.I. Nos., Canuck (aBCd) and Markton (abcd) to oat stem rust race 6.

The resistance of these eight entries to race 13A (Fig. 3) is of especial interest. This race was described from 1957 collections from Quebec and New York as attacking all known sources of resistance among hexaploid oats except Jostrain (1, 5) (Fig. 4). Jostrain, a stem rust differential variety, gives resistance of a mesothetic type to race 13A. However, Jostrain is known to be unstable for rust reaction and has been of little value in breeding programs as a source of stem rust resistance.



2413 2710 3030 3031 3037 3038 3039 3040 aBCd abcd
Oat Stem Rust Race 13A

FIGURE 3. Seedling reactions of eight C.I. Nos., Canuck (aBCd) and Markton (abcd) to oat stem rust race 13A.



Abcd abcD aBCd AbcD aBCD ABCd ABCD 7-0-3 abcd
Oat Stem Rust Race 13A

FIGURE 4. Seedling reactions to stem rust race 13A of oat varieties containing known stem rust resistance genes in different combinations, and variety Sevnothree (7-0-3). See legend, Figure 2.

Thus C.I. 2413, 2710, 3030, 3031, 3037, 3038, 3039, and 3040 would seem to promise not only resistance to races 7, 7A, and 8, but especially to races 6 and 13A. The pathologic reactions of these oat lines to these races of the oat stem rust fungus indicate that they may contain one or more new genes for stem rust resistance.

Literature Cited

1. ANONYMOUS. 1958. Stem rust of oats. In Canada Dept. Agr., Cereal Crops Div., Cereal News 3 (4): 14.
2. BAILEY, DIXON L. 1925. Physiologic specialization in Puccinia graminis avenae Erikss. and Henn. Minnesota Agr. Exp. Sta. Tech. Bul. 35: 1-33.
3. GORDON, W. L. 1928. Physiologic forms of Puccinia graminis avenae Erikss. and Henn. in Canada. Sci. Agr. 8: 462-463.
4. GORDON, W. L., and D. L. BAILEY. 1928. Physiologic forms of oat stem rust in Canada. Sci. Agr. 9: 30-38.
5. GREEN, G. J., B. PETURSON, and D. J. SAMBORSKI. 1958. Cereal rusts in Canada in 1957. Canada Dept. Agr., Plant Pathology Sect., Rept. 13: 23-25.
6. KEHR, W. R., H. K. HAYES, M. B. MOORE, and E. C. STAKMAN. 1950. The present status of breeding rust resistant oats at the Minnesota Station. Agron. Jour. 42: 356-359.
7. NEWTON, MARGARET, and T. JOHNSON. 1944. Physiologic specialization of oat stem rust in Canada. Can. J. Res. Sec. C, 22: 201-216.
8. RODENHISER, H. A., and H. C. MURPHY. 1955. Reaction to crown and stem rust of oat varieties and selections in uniform nursery grown in South America in 1953-54. Agr. Res. Service, U. S. Dept. Agr., ARS-345cc, 21 p.
9. STAKMAN, E. C., D. M. STEWART, E. B. HAYDEN, and B. J. ROBERTS. 1955. Physiologic races of Puccinia graminis in the United States in 1954. Agr. Res. Service, U. S. Dept Agr., ARS-81-3, 15 p.
10. STEWART, D. M., R. U. COTTER, B. J. ROBERTS, and E. B. HAYDEN. 1956. Physiologic races of Puccinia graminis in the United States in 1955. Plant Dis. Reptr. Suppl. 239: 99-105.
11. STEWART, D. M., R. U. COTTER, and B. J. ROBERTS. 1957. Physiologic races of Puccinia graminis in the United States in 1956. Plant Dis. Reptr. Suppl. 245: 53-60.
12. WELSH, J. N. 1937. The synthetic production of oat varieties resistant to race 6 and certain other physiologic races of oat stem rust. Can. J. Res. Sec. C, 15: 58-69.
13. WELSH, J. N., and T. JOHNSON. 1951. The source of resistance and the inheritance of reaction to 12 physiologic races of stem rust, Puccinia graminis avenae (Erikss. and Henn.). Can. J. Bot. 29: 189-205.
14. WELSH, J. N., and T. JOHNSON. 1954. Inheritance of reaction to race 7A and other races of oat stem rust, Puccinia graminis avenae. Can. J. Bot. 32: 347-357.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, IOWA STATE COLLEGE, AMES, IOWA

EFFECT OF SEED TREATMENTS WITH ANTIBIOTICS ON BACTERIAL WILT OF CORN[1]

John J. Natti

Summary

Soak treatments of corn seed in water solutions of anisomycin, streptomycin nitrate, and streptomycin sulfate provided varying degrees of control of bacterial wilt in greenhouse tests. Tyrothricin appeared to provide slight protection. Endomycin, filipin, fungichromin, griseofulvin, mycostatin, neomycin sulfate, polycycline, thiactin, and thiolutin were completely ineffective. Storage of treated seeds in a dry condition as long as 270 days had no apparent effect on the seeds or on the effectiveness of the test materials in controlling bacterial wilt. Seed-soak treatments with anisomycin, streptomycin nitrate, and streptomycin sulfate caused bleaching of the foliage of the corn seedlings. The bleaching action of streptomycin was not prevented by retreatment of soaked seeds with fungicides. None of the treatments was sufficiently effective to be considered of practical importance in controlling bacterial wilt. The treatments which provided control were injurious to the seedlings.

Bacterial wilt incited by Bacterium stewartii E. F. Sm. is a destructive disease of seedlings of susceptible varieties of sweet corn. Corn seedlings infected within several weeks of emergence usually die or remain stunted and unproductive. Plants which become infected later in the season, however, generally produce marketable ears. Protection of corn seedlings from bacterial wilt infection during the seedling stage, therefore, would be of economic importance, particularly to the grower of early sweet corn for fresh market purposes. In greenhouse tests, Rich (1) and Williams (2) observed that the incidence and severity of bacterial wilt were less among seedlings originating from seeds soaked in solutions or suspensions of various compounds, including antibiotics, than among corn seedlings from untreated seeds. Williams (2) observed that, in general, the most effective treatments were also the most phytotoxic. In the present study a number of antibiotics and methods of treatment of corn seeds were tested in an attempt to develop a treatment which would provide effective control of bacterial wilt without causing injury to the corn seedlings.

MATERIALS AND METHODS

Seeds of Seneca Arrow sweet corn were used in all tests. Seed-soak treatments were applied by submerging 50 gm of seeds in 500 ml of a water solution or suspension of the test material for a prescribed time. The seeds were then dried on paper towels. Dust and slurry treatments were applied by adding the required amount of test material to 50 gm of seeds in a glass jar and then slowly rotating the jar on a motor-driven wheel for 3 minutes. The seeds in some tests were planted within 24 hours of treatment. Other seeds were maintained for various lengths of time in the laboratory to determine whether storage of the seeds in a treated condition had any adverse effects on the seeds or had any influence on the effectiveness of the treatments. All seeds were treated and stored in the laboratory.

A 24-hour old culture of Bacterium stewartii grown on nutrient dextrose agar in a 1000-ml Roux flask and suspended in 500 ml of distilled water was used as inoculum. The inoculum was used within one-half hour after being prepared.

The treatments were evaluated for control of bacterial wilt in the greenhouse. The seeds were planted in composted soil in 4-inch pots, three seeds per pot. The seedlings were inocu-

---

[1] Approved by the Director as Journal Paper No. 1134. New York State Agricultural Experiment Station, Geneva, New York, June 28, 1958.

Table 1. Control of bacterial wilt of corn seedlings and incidence of bleached plants from seed-soak treatments with streptomycin sulfate and streptomycin nitrate.

Treatments [a]	Conc. ppm (actual)	No. plants inoculated	No. plants infected	No. plants bleached [b]
Streptomycin sulfate (25%)	800	45	10	29
	400	44	10	24
	200	45	19	12
	100	47	29	4
Streptomycin nitrate (20%)	800	48	16	31
	400	48	22	27
	200	48	38	5
	100	47	40	0
Water (24-hour soak)	---	43	39	0
Dry Seeds	---	45	44	0
Water (24-hour soak, not inoculated)		46	0	0
Dry Seeds (not inoculated)		47	0	0

[a] 48 seeds per treatment were soaked in antibiotic solution for 17 hours; seeds planted 24 to 26 hours after completion of soak treatments.

[b] Includes all gradations of bleaching from slight to complete.

lated at the three-leaf stage of growth by injecting inoculum into the base of the stem by means of a hypodermic needle and syringe. The seedlings were examined for symptoms of bacterial wilt 16 days after inoculation. Because the phytotoxic effects of some of the treatments would have interfered with the rating of plants for severity of bacterial wilt, data were taken only on the incidence of infection.

Treatments

Experiment 1: The effectiveness of seed-soak treatments with streptomycin sulfate and streptomycin nitrate on control of bacterial wilt of corn seedlings was compared. The seeds were soaked for 17 hours in solutions of the test materials which contained streptomycin at concentrations of 100, 200, 400, and 800 ppm. Forty-eight seeds from each treatment were planted 22 to 24 hours after the treatments were completed.

Experiment 2: The influence of concentration of streptomycin and duration of seed-soak treatment on control of bacterial wilt and on phytotoxicity to corn seedlings was determined. Seeds were soaked for 1, 6, 24, and 48 hours in solutions of streptomycin sulfate containing streptomycin at concentrations of 312, 625, 1250, 2500, and 5000 ppm. The pericarp of a portion of the seeds was nicked to facilitate the entrance of the test material into seeds. Twenty-one seeds from each treatment were planted 5 days after treatment.

Experiment 3: Seeds subjected to soak treatments in solutions of streptomycin sulfate were retreated with seed protectants to determine whether the phytotoxic effects of streptomycin could be eliminated or reduced by fungicides applied to the surface of the seeds. Corn seeds were soaked for 24 hours in the test solutions, which contained streptomycin at concentrations of 200 and 500 ppm. Twenty-four hours after the soak treatments were completed, portions of this seed were retreated with Arasan (75%) dust, Orthocide 75 dust, and with slurry preparations of Delsan A-D and Ortho Seed Guard. Other lots of the same seeds which had not been given soak treatments were treated with streptomycin dust at the rate of 2 ounces of streptomycin per bushel of seeds. Streptomycin dust was also suspended in a 2% Methocel solution and applied as slurry treatments to the seeds. Forty-eight seeds from each treatment were planted 48 to 52 hours after the soak treatments were completed.

Experiment 4: Seed-soak treatments with a number of antibiotics known to have antifungal activity were tested to determine whether these materials would be of value in providing control of both bacterial wilt and of seed decay. Intact seeds and nicked seeds were soaked for 1,

Table 2. Influence of concentration of streptomycin and duration of seed-soak treatment on control of bacterial wilt of corn seedlings.

Conc. of STS (ppm) [a]	Duration of seed soak (hour)	Intact Seeds		Nicked Seeds [b]	
		No. plants inoculated	No. plants infected	No. plants inoculated	No. plants infected
5000	1	17	16	16	12
	6	14	10	14	10
	24	9	2	7	0
	48	(no plants) [c]		(no plants)	
2500	1	19	17	18	15
	6	15	14	15	14
	24	19	6	11	1
	48	(no plants)		(no plants)	
1250	1	17	14	19	16
	6	15	13	14	12
	24	15	8	12	1
	48	10	2	(no plants)	
625	1	19	16	19	16
	6	16	13	16	12
	24	13	5	12	3
	48	8	1	(no plants)	
312	1	19	14	19	16
	6	19	16	18	16
	24	13	11	9	6
	48	9	2	8	4
Water	1	20	17	19	17
	6	18	16	18	17
	24	16	15	17	15
	48	18	17	17	16
Dry Seeds	A	20	18	19	17
	B	20	18	21	18
	C	21	18	19	17
	D	20	19	20	19
Water (not inoc.)	1	18	0	21	0
	6	19	0	20	0
	24	19	0	21	0
	48	20	0	19	0
Dry Seeds (not inoc.)		19	0	20	0

[a] STS = streptomycin sulfate, 50% pure blend; ppm indicates concentration of active ingredient.
[b] Pericarp of seeds nicked with a file.
[c] Plants either failed to emerge or died soon after emergence.

3, and 6 hours in dilutions of the various antibiotics which contained the active ingredient at concentrations of 500, 1000, and 2000 ppm. Twelve seeds from each treatment were planted 7, 90, 150, and 270 days after treatment. Data were taken on control of bacterial wilt and phytotoxicity but not on control of seed decay.

RESULTS

The incidence of bacterial wilt among corn seedlings originating from seeds subjected to soak treatments in solutions of streptomycin sulfate and streptomycin nitrate was less than that among corn seedlings from untreated seeds (Table 1). Streptomycin sulfate appeared to be more effective than streptomycin nitrate. Seed-soak treatments at concentrations of 400 and 800 ppm were more effective in protecting seedlings from wilt than treatments at 100 and 200 ppm. Seed

Table 3. Control of bacterial wilt of corn seedlings and incidence of bleached plants from seed treatments with streptomycin alone and in combination with seed protectants.

Seed Treatments [a]	No. plants Inoculated	No. plants Infected	No. plants Bleached
STS 500 ppm; soak (24 hours)	40	28	21
" 200 " "	43	31	4
" 500 " " ; retreated with Arasan (dust)	39	24	17
" 200 " " " " " "	43	29	5
" 500 " " " " Orthocide 75 (dust)	41	29	20
" 200 " " " " " "	45	31	7
" 500 " " " " Delsan A-D (slurry)	45	28	18
" 200 " " " " " "	44	39	4
" 500 " " " " Ortho Seed Guard (slurry)	45	23	14
" 200 " " " " " " " "	41	35	5
" 2 oz./bu. (dust)	45	40	2
" " in 2% Methocel solution (slurry)	43	31	3
" " plus Arasan (dust)	45	42	0
" " " Orthocide 75 (dust)	44	43	0
" " " Arasan in 2% Methocel solution (slurry)	42	32	3
" " " Orthocide 75 in 2% Methocel solution (slurry)	43	31	2
Water; soak (24 hours)	45	42	0
No treatment	44	42	0
Water; soak (24 hours); not inoculated	44	0	0
No treatment; not inoculated	44	0	0

[a] STS = streptomycin sulfate (50% purified blend); Arasan and Orthocide 75 used at rate of 1.3 oz./bu. of seed; Delsan A-D and Ortho Seed Guard used at rate of 1.5 oz./bu. Slurry treatments applied at rate of 1/2 pint per bu. of seed.

soaks at the higher dosages also caused considerable bleaching of the foliage. In general, bleached seedlings did not become infected.

Seed-soak treatments of 1 hour duration in solutions of streptomycin sulfate containing streptomycin at concentrations as high as 5000 ppm were ineffective in protecting seedlings from bacterial wilt (Table 2). Seed-soak treatments of 6 hours duration provided slight protection at all dosages. Seed-soak treatments of 24 hours duration provided a high degree of protection at all but the lowest dosage. However, many of the seedlings which did not become infected were severely bleached and died several weeks after data on disease incidence were taken. Seed soaks of 48 hours duration were injurious at all dosages. Some of the seeds had germinated upon the completion of the 48-hour soak period. Only ungerminated seeds were planted. The incidence of wilt was the same from nicked seed as from intact seed.

The control of bacterial wilt and the phytotoxic effects of streptomycin on corn seedlings originating from seed subjected to soak treatments in solutions of streptomycin sulfate was not affected by retreatment of the seeds with dust applications of Arasan or Orthocide 75, or with slurry treatments of Delsan A-D or Ortho Seed Guard (Table 3). Dust applications of streptomycin alone or of streptomycin combined with Arasan dust and Orthocide 75 dust were ineffective in protecting seedlings from bacterial wilt. These dusts suspended in 2% Methocel provided slight protection. Dust and slurry applications of streptomycin to corn seed were not so effective in providing protection against bacterial wilt as were seed-soak treatments.

In tests with different antibiotics in which the seeds were planted 7 days after treatment, 35 percent of the inoculated seedlings from all treatments with streptomycin remained healthy (Table 4). Many of the seedlings were bleached. Anisomycin provided protection comparable to that given by streptomycin and caused bleaching and stunting of the corn seedlings. Polycycline was ineffective, reduced germination, and caused stunting of the seedlings. Tyrothricin reduced the incidence of wilt in some instances but generally was ineffective. Endomycin, filipin, fungichromin, griseofulvin, mycostatin, neomycin sulfate, thiactin, and thiolutin were completely ineffective and had no adverse effects on the seeds or seedlings under the conditions of this test. Seedlings from nicked seeds were generally more severely injured than those

Table 4. Bacterial wilt control and severity of injury to corn seedlings from seed planted 7 days after seed-soak treatments with antibiotics.

Antibiotic [a] and conc. (ppm)	Seed soak (hours)	Intact Seeds			Nicked Seeds		
		No. plants inoculated	No. plants infected	Injury rating [b]	No. plants inoculated	No. plants infected	Injury rating
Strep. sulfate							
2000	1	12	5	+++	12	7	+++
"	3	11	5	+++	12	8	+++
"	6	11	6	+++	10	5	+++
1000	1	11	10	++	12	10	++
"	3	11	7	+++	11	6	+++
"	6	11	6	+++	10	5	+++
500	1	12	10		12	9	+
"	3	11	8	+	11	8	++
"	6	10	9	+	11	6	++
Anisomycin							
2000	1	10	5	+++	8	5	+++
"	3	12	7	+++	7	3	+++
"	6	8	6	+++	8	4	+++
1000	1	10	6		9	7	+
"	3	11	8		11	9	++
"	6	10	9	+	10	8	++
500	1	11	11		9	6	
"	3	12	10		10	8	
"	6	10	10		11	10	+
Polycycline							
2000	1	11	11	+++	8	8	+++
"	3	12	11	+++		(no plants) [c]	
"	6	12	12	+++		(no plants)	
1000	1	12	12	+++	11	11	
"	3	12	12	+++	11	10	+++
"	6	10	10	+++	6	6	+++
500	1	10	10		11	11	+
"	3	12	12	++	11	11	+++
"	6	10	10	+++	12	12	+++
Tyrothricin							
2000	1	10	8		11	7	
"	3	10	8		9	7	
"	6	11	10		10	10	
1000	1	11	11		11	9	
"	3	10	10		12	10	
"	6	11	11		12	12	
500	1	10	8		11	7	
"	3	11	9		11	10	
"	6	12	12		12	12	
Water	1	11	11		11	11	
	3	11	11		12	11	
	6	12	11		12	12	
Dry Seeds	-	11	11		12	12	
Water (not inoculated)	1	11	0		12	0	
	3	12	0		12	0	
	6	11	0		11	0	
Dry Seeds (not inoculated)	-	11	0		10	0	

[a] The following antibiotics provided no control of bacterial wilt and were noninjurious under the conditions of this test: endomycin, filipin, fungichromin, griseofulvin, mycostatin, neomycin sulfate, thiactin, and thiolutin.

[b] Injury rating = (+) slight; (++) = moderate; and (+++) = severe.

[c] Seedlings failed to emerge or died soon after emergence.

from intact seeds. Nicking had no effect on disease control.

Results from tests in which the seeds were planted 90 and 270 days after treatment were essentially the same as those from the test in which the seeds were planted 7 days after treatment. Streptomycin sulfate and anisomycin provided fair protection and caused injury to the seedlings even after the extended period of storage of the seeds in a treated condition. The germination of seeds from the polycycline treatments which had been stored for 270 days was about 50 percent less than that of seeds planted 7 days after treatment. The treatments with the other antibiotics had no apparent effect on control of bacterial wilt or on the seeds or seedlings. In the test on seeds planted 150 days after treatment, most of the inoculated plants failed to become infected, even those from untreated seeds, probably because of excessively high greenhouse temperatures at the time of inoculation.

DISCUSSION

No material or method of treatment of corn seeds with antibiotics provided effective control of bacterial wilt without causing injury to the corn seedlings. However, the results demonstrated that materials absorbed by corn seeds are capable of preventing infection of corn seedlings with bacterial wilt. Whether the treatments make the seedlings resistant as suggested by Rich (1) or whether the material absorbed by the seed is translocated into the developing seedlings and serves as a systemic protectant was not determined. Storage of seeds in a treated condition as long as 270 days did not influence the effectiveness of the treatments nor adversely affect the seeds.

The bleaching of corn seedlings from treatments with streptomycin resulted from the inhibition of chlorophyll development. Spectrophotometric determinations indicated that chlorophyll and carotene were entirely absent in seedlings which were completely bleached. Bleached seedlings failed to become pigmented even when exposed to sunlight. Plastids which appeared to be normal in size but completely devoid of chlorophyll or starch were observed in epidermal strips from bleached foliage. Plastids devoid of chlorophyll along with apparently normal chloroplasts were observed in epidermal strips from partially bleached seedlings. These observations suggest that the bleaching of foliage of certain plants sprayed with streptomycin is not caused by the destruction of the already formed chloroplasts but rather by the inhibition of synthesis of chlorophyll.

The bleaching action of streptomycin on corn seedlings was not uniform. In some instances, seedlings from seeds soaked in dilute solutions of streptomycin were more severely bleached than were seedlings from seeds soaked in more concentrated solutions. At higher concentrations of streptomycin, some plants were completely bleached while others appeared to be normal. This nonuniformity of bleaching of seedlings was present even among seedlings from seeds which were nicked to facilitate the absorption of the test material by the seeds.

Literature Cited

1. RICH, SAUL. 1956. Seed treatments to protect corn seedlings against Stewart's wilt. Plant Disease Reptr. 40: 417-420.
2. WILLIAMS, LANSING E. 1957. Effects of some materials on Stewart's bacterial wilt of sweet corn when applied as seed treatments. Plant Disease Reptr. 41: 919-922.

DEPARTMENT OF PLANT PATHOLOGY, NEW YORK STATE AGRICULTURAL EXPERIMENT STATION, GENEVA, NEW YORK

ANTIBIOTIC PROPERTIES OF THE SEEDS OF WHEAT AND BARLEY

Peter A. Ark[1] and J. P. Thompson[2]

Abstract

Seeds of both barley and wheat contain an antibiotic principle active against Gram-positive bacteria and fungi when tested in vitro. The antibiotic principle is extractable with water and methyl alcohol and is not destroyed either by lyophilization, air drying, or partial autoclaving.

Several workers (1, 3, 9) have reported that control of loose smut of barley can be achieved to a certain degree by soaking the seed in water for a specified period of time. It is suggested that the effect may be due to the anaerobic fermentation. Zaleskii (14) reported a good control of Ustilago tritici on wheat by anaerobic fermentation. Winter (13) indicated a demonstrable antibiotic in certain species of Gramineae, and Stiven (6) reported on the production of antibiotic substances by the roots of the grass Trachypogon plumosus. Novotelnov (4, 5), working with wheat, barley, oats, and rye, showed that the outer layer of these grains contained an antibiotically active chemical belonging to the flavones and active only against the Gram-negative bacteria.

The present paper deals with antibiotic effects of seeds of wheat and barley against plant pathogens and other organisms.

MATERIALS, PROCEDURES, AND RESULTS

In one series of experiments, the seeds of known varieties of wheat and barley[3] were extracted with distilled water or absolute methyl alcohol in the ratio of one part seed to two parts of the extractant. Standard bio-assay filter paper discs (S-S, 740E), measuring about 13 mm in diameter, were dipped to saturation, air dried for 20 minutes and placed on plates of agar media previously inoculated by flooding with the test organisms. The plates were incubated at 28° C for 24 and 48 hours and read for inhibition zones with the antibiotic zone reader. In another series of tests whole seeds of wheat were placed on the incubated plates and thereafter treated as in the first series.

Since no activity could be detected against many Gram-negative bacteria in any of the writers' tests, only Gram-positive organisms were included in the experiments, namely, Corynebacterium michiganense, Micrococcus flavus, Bacillus subtilis, B. mycoides, and Mycobacterium phlei. An antibiotic principle active against all tested Gram-positive organisms was detected in various degrees of strength from both wheat and barley seeds. Of the fungi the following organisms showed definite but different degrees of sensitivity to the antibiotic principle contained in the experimental seeds: Verticillium albo-atrum, Fusarium oxysporum f. phaseoli, F. solani f. cucurbitae, Cladosporium cucumerinum, Aspergillus niger, Thielaviopsis basicola, Saccharomyces cerevisiae, Monilinia fructicola, Glomerella cingulata, Uromyces phaseoli, Pseudoperonospora cubense, and Streptomyces scabies.

Table 1 shows the zones of inhibition produced on plates of Corynebacterium michiganense by aqueous and methyl alcohol extracts of various varieties of the wheat and barley seed. It is interesting to note that the antibiotic principle is not exhausted by repeated 24-hour extractions with either methyl alcohol or distilled water. The zones of inhibition on plates of C. michiganense for six consecutive extractions of the wheat seed were as follows (in mm): 35. 8, 32, 27, 26, 26, and 27. Lyophilization does not seem to destroy the antibiotic principle and 4 days of air drying the saturated discs did not diminish the antibiotic. Sterilization of the moistened wheat seed at 15 pounds pressure for 10 minutes did not inactivate the antibiotic principle contained in it.

---

[1] Professor of Plant Pathology, University of California, Berkeley, California.

[2] Laboratory Technician II.

[3] The authors are grateful to Dr. Philip M. Halisky of the Department of Plant Pathology, University of California, Davis, California, for furnishing some of the varieties of both barley and wheat used in the experiments.

Table 1. Zones of inhibition (diameter in mm) produced by aqueous and methyl alcohol extracts of different varieties of wheat and barley against Corynebacterium michiganense on potato-dextrose-peptone agar. Penicillin assay paper discs (S & S. 740 E) filter paper (13 mm diameter) were dipped into the extracts, dried in air for 20 minutes and placed on inoculated plates. Incubation 48 hours at 28° C. Read by the antibiotic zone reader.

Wheat variety	Zones of inhibition (diameter in mm): Aqueous extract	Zones of inhibition (diameter in mm): Methyl alcohol extract	Barley variety	Zones of inhibition (diameter in mm): Aqueous extract	Zones of inhibition (diameter in mm): Methyl alcohol extract
Baart 46	42	40	Atlas	19	17
Big Club 43	31	29	Atlas 46	15	14
Bunyip 41	23	22	Arivat	32	32
Onas 53	18	17	California Mariot	37	36
Pacific Blustem 37	20	14	Club Mariot	14	14
Seneca	13	13	Rojo	21	13
White Federation 54	20	17	Vaughn	40	29
----	--	--	Unknown	45	43

Figure 1 shows inhibition zones produced by aqeous extracts of the wheat seed against both bacteria and fungi. In Figure 2 the zones of inhibition are those produced by slightly moistened seeds of wheat, var. Baart 46, against Corynebacterium michiganense. The average size of the zones produced by Baart 46 seeds is about 27 mm. One wheat variety, Seneca, and one barley variety, Club Mariot, had a low antibiotic content.

DISCUSSION

The existence of antibiotics in higher plants has been known to scientists for some time. However, its role as a possible defense mechanism from incursions by plant or animal elements in the immediate proximity of the plant has not received a satisfactory degree of scientific inquiry and practical utilization. Grümmer (2) and Tokin (7, 8) emphasize naturally-occurring antibiotics in higher plants as contributing factors of natural immunity in plants and state that these factors can be of either solid or volatile nature. In the light of such premise it seems natural to propose an explanation that a certain degree of control of loose smut of barley and wheat (9), or the control of Rhizopus sp. on barley seed by propylene oxide if water is added to the seed before addition of the propylene oxide, as was recently reported by Tyner (10), can be attained. It also is of interest to note that in the writers' experiments with germinating wheat, the hypocotyl and young roots were just as active antibiotically as the seeds. The experiments were not carried far enough to determine if the antibiotic principle main tained the same level throughout the growth of the plant.

Naturally-occurring antibiotics present very interesting possibilities. Thus, Virtanen (12) and Valle (11) in their recent papers reported the isolation of 2(3)-benzo-xazolinone and chlorogenic acid from rye and potato leaves respectively, and were able to correlate the substances with resistance to Fusarium nivale and Phytophthora infestans, respectively. The isolated substances occur naturally in the hosts, according to the investigators. The fact that these naturally-occurring, strongly antibiotic materials can be synthesized in the laboratory without great difficulty may point the way to a very profitable and rewarding research in solving certain difficult plant pathological problems.

CONCLUSION

Seeds of wheat and barley of different varieties, when moistened and placed on test plates seeded with Gram-positive plant pathogenic and saprophytic bacteria, show considerable zones of microbial inhibition around them after 24-hour incubation at 28° C.

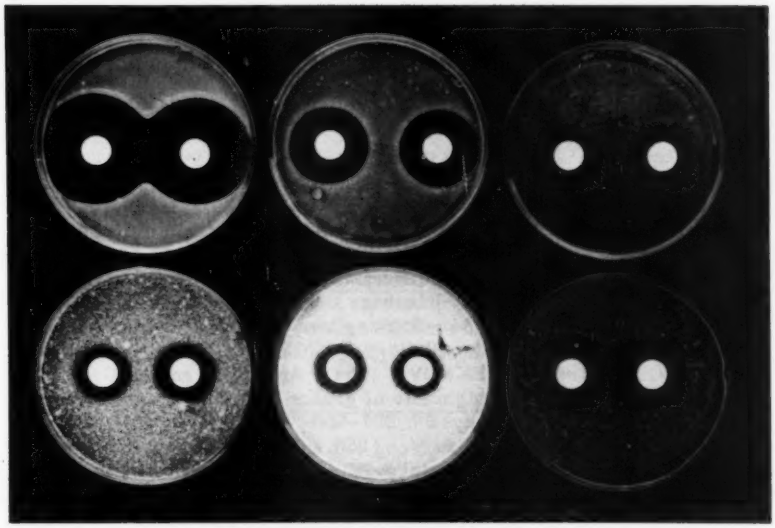


FIGURE 1. Inhibition zones formed by an aqueous extract of wheat seed, var. Baart 46. Upper row, left to right, Corynebacterium michiganense, Micrococcus flavus, Thielaviopsis basicola. Bottom row, left to right: Fusarium solani f. cucurbitae, Verticillium albo-atrum, and Mycobacterium phlei.

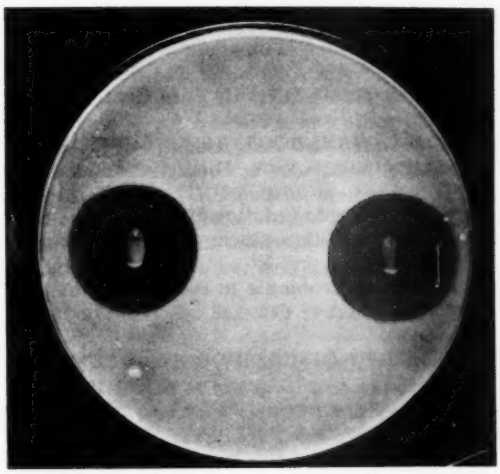


FIGURE 2. Inhibition zones caused by a diffusate from the presoaked wheat seeds (Baart 46) on agar plates of Corynebacterium michiganense. Incubation: 24 hours at 28° C.

Aqueous and methyl alcohol extracts of both barley and wheat seeds showed strong antibiotic action when bioassayed against both the bacteria (Gram-positive) and the fungi. Water extracts were more active.

Neither lyophilization or air drying (4 days) inactivated the antibiotic principle contained in aqueous extracts and seeds of barley and wheat.

Content of the antibiotic principle ranged from none in wheat, var. Seneca, to a very considerable quantity as in the variety Baart 46.

Literature Cited

1. ARNY, D. C., and C. LEBEN. 1956. Control of several grain diseases by the water-soak treatment. Phytopathology 46: 344-345.
2. GRÜMMER, G. 1955. Die gegenseitige Beeinflüssung höheren Pflanzen. Allelopathie. 162 p. Vel Gustav Fischer, Jena.
3. LEBEN, C., and D. C. ARNY. 1954. Soaking treatment for the control of loose smut of barley. Phytopathology 44: 329-330.
4. NOVOTELNOV, N. V., and I. S. EZHOV. 1954. Antibiotic and antioxydant properties of yellow pigments of grains. Compt. Rend. Acad. Sci. S. S. S. R. (Doklady) 99: 297-300.
5. NOVOTELNOV, N. V., and I. S. EZHOV. 1956. O videlenii antibioticheskikh veschestv zernamy zlakovikh kultur. Trudy Leningr. Technolog. Instituta Kholodnoi Promi-Shlennosty 14: 179-186.
6. STIVEN, G. 1952. Production of antibiotic substances by roots of grass (Trachypogon plumosus (HBK) Mes.) and of Pentamisia variabilis (E. Meg.) Harv. (Rabiaceae). Nature (London) 170: 712-713.
7. TOKIN, B. P. 1956. Phytoncide. 230 p. Vel Verblag Volk und Gesundheit, Berlin.
8. TOKIN, B. P. 1957. Phytoncidy i ikh rol' v prirode. 225 p. Leningrad University, Leningrad, U. S. S. R.
9. TYNER, L. E. 1953. The control of loose smut of barley and wheat by Spergon and by soaking in water at room temperature. Phytopathology 43: 313-316.
10. TYNER, L. E. 1958. The effect of water on the partial sterilization of barley seed by propylene oxide and by heat. Phytopathology 48: 177-178.
11. VALLE, E. 1957. On antifungal factors in potato leaves. Acta Chem. Scand. 11: 395-397.
12. VIRTANEN, A. I., P. K. HIETALA, and Ö. WAHLROOS. 1957. Antimicrobial substances in cereals and fodder plants. Arch. Bioch. and Biophysics 69: 486-500.
13. WINTER, A. G., and LISEL WILLEKE. 1952. Antibiotics from higher plants. V. Inhibitors in leaves and straw of Gramineae. Naturwissenschaften 39: 190-191.
14. ZALESKII, V. K. 1935. A new method for anaerobiosis in the control of Ustilago tritici on wheat. Zaschita Rastenii 1: 135-138.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY 4, CALIFORNIA

EFFECT OF GIBBERELLIC ACID ON DAMPING-OFF OF LADINO WHITE CLOVER

J. H. Graham[1]

Abstract

Gibberellic acid applied at 10 and 40 ppm to seed of Ladino white clover increased the amount of pre-emergence damping-off in soil infested with Pythium debaryanum. Postemergence damping-off was increased significantly by gibberellic acid in soil infested with each of the following pathogens: P. debaryanum, Rhizoctonia solani, and Fusarium roseum. Since the chemical had no measurable effect on the three fungi in culture, it is assumed that the altered growth of the seedlings (spindly and light green) increased their susceptibility to the damping-off organisms.

Since gibberellic acid increases the growth rate of many plants, it was postulated that the time during which seedlings of Ladino white clover remain in the damping-off stage might be shortened by treatment with the chemical and both pre- and postemergence damping-off be reduced thereby. To test this theory two greenhouse experiments were conducted, using three fungi and two rates of gibberellic acid[2] plus controls.

Soil in metal pans was steamed for 6 hours at approximately 5 pounds pressure. After the soil had cooled, mycelial suspensions of each fungus, Fusarium roseum (Lk.) Snyder & Hansen, Rhizoctonia solani Kuehn, and Pythium debaryanum Hesse, were mixed with the top 3 inches of soil in two pans. An equal amount of water was mixed with soil in two pans as a control. In each pan, nine rows 1/4 inch deep were seeded to Pilgrim Ladino white clover at the rate of 100 seeds per row. The seed had a germination rate of 97 percent (26 percent hard seed). Before covering the seed, three treatments (three rows per treatment), consisting of 10 ml of gibberellic acid solution at the concentrations of 10 and 40 ppm, and of water, were pipetted on the seed. After the seed had been covered, 5 ml of each of the above treatments were added to the respective rows.

Greenhouse temperatures ranged from 18° to 23° C. Watering was regulated to favor each pathogen as learned in a previous study[3]. The present experiment involving two replications (6 rows) was conducted in November 1957 and repeated in December 1957.

Seedling counts were made every other day and damped-off seedlings were removed. Data were recorded as total emergence and postemergence damping-off.

Adjunct experiments were conducted to determine the effect of gibberellic acid on the three fungi in culture. Gibberellic acid at 10 ppm and 40 ppm, and a water control, were added to warm potato-dextrose agar immediately before pouring into Petri dishes. After the agar had solidified, 4-mm discs of each fungus were placed in each plate and incubated at 23° C. Two tests, one with three and one with four replications, were conducted.

RESULTS

Neither the rate of mycelial growth nor the characteristics of the colonies were affected by the gibberellic acid at the two rates used.

Results of the seedling experiments are presented in Tables 1 and 2. In steamed soil the rate of emergence of seedlings from gibberellic acid-treated seed was higher and the seedlings were taller (longer petioles) and lighter green than those from non-treated seed. In the first test, final emergence in steamed soil was the same from treated and untreated seed; in the second test, however, the rate of emergence (and number emerged) of seedlings from seed receiving no gibberellic acid remained behind the treated lots throughout the experiment.

---

[1]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, United States Regional Pasture Research Laboratory, University Park, Pennsylvania.

[2]Used commercial preparation Gibrel, courtesy of Merck & Co., Inc.

[3]Graham, J. H., V. G. Sprague, and R. R. Robinson. 1957. Damping-off of Ladino clover and Lespedeza as affected by soil moisture and temperature. Phytopathology 47: 182-185.

Table 1. Percentage emergence of Ladino white clover as affected by fungi and gibberellic acid.

Fungus	Percentage emergence with indicated amount of gibberellic acid					
	None		10 ppm		40 ppm	
	Tests		Tests		Tests	
	I	II	I	II	I	II
Control	66[a]	58	66	64	68	64
Fusarium	47	61	44	64	41	60
Pythium	44	43	35	31	32	32
Rhizoctonia	33	65	32	66	35	66

[a]Represents 6 rows, 100 seeds planted per row. LSD, 0.05: Fungi 5.5; Fungi x Tests 7.9; Concentration x Fungi 5.3.

Table 2. Percentage postemergence damping-off of Ladino white clover as affected by fungi and gibberellic acid.

Fungus	Percentage damping-off from indicated treatment with gibberellic acid					
	None		10 ppm		40 ppm	
	Tests		Tests		Tests	
	I	II	I	II	I	II
Control	2.3[a]	3.2	2.5	6.0	2.0	6.6
Fusarium	11.0	16.1	32.0	29.1	31.7	28.0
Pythium	14.8	16.8	25.0	44.2	25.4	41.4
Rhizoctonia	21.8	15.3	29.5	30.3	35.4	22.9

[a]Represents 6 rows, 100 seeds planted per row. LSD, 0.05: Fungi 8.4; Concentrations 3.6; Concentrations x Fungi 6.9.

Of the fungi, only Pythium reduced total emergence significantly in both tests. In addition, in soil infested with Pythium, emergence was adversely affected by the addition of 10 ppm and 40 ppm of gibberellic acid. The analysis of variance indicated that concentration effects were not significant, owing to the opposite effects of gibberellic acid in steamed soil and in Pythium-infested soil. Since the concentrations x fungi interaction was significant at the 0.05 level, it appears that the true differences due to gibberellic acid concentration were cancelled.

Postemergence damping-off was significantly greater in soil infested with each of the three fungi than in the control. Significantly more seedlings damped- off in infested than in non-infested soil when seeds were treated with 10 ppm and 40 ppm of gibberellic acid. There were no differences in the effects of the two rates of gibberellic acid.

Since gibberellic acid had no measurable effect on these fungi in culture, it is assumed that the altered growth of the seedlings (spindly and light green in color) increased their susceptibility to the damping-off organisms.

These results indicate that gibberellic acid increases the proclivity of Ladino white clover seedlings to damping-off instead of reducing seedling loss.

FORAGE AND RANGE RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, UNITED STATES REGIONAL PASTURE RESEARCH LABORATORY, UNIVERSITY PARK, PENNSYLVANIA

STREPTOMYCIN-FUNGICIDE MIXTURES AS POTATO SEED PIECE TREATMENTS[1]

Harry C. Fink

Streptomycin was introduced as a potato seed piece treatment by Bonde (1, 2, 3) who reported it effective against bacterial rots. Fink et al. (4), Waggoner (5), and Palm and Young (6) reported on the use of fungicide-streptomycin mixtures and their effect on yield. Because in some cases yields were increased with the use of mixtures while in other cases the same mixtures resulted in lowered stands and yields, a laboratory and field study was initiated at Pennsylvania State University to try to determine if fungicide-streptomycin mixtures are effective against seed piece rot due to Erwinia atroseptica, the "black leg" pathogen.

METHODS AND MATERIALS

Field Tests: All field tests were conducted on the University Farm at University Park, Pennsylvania. The field was of uniform soil type identified as Hagerstown silt loam and had been in potatoes for 3 years. Only one variety of potato, Katahdin, was used. Seed pieces were cut with an electric seed piece cutter and each seed piece was examined to determine if it contained a "bud eye" before it was accepted for the test. Seed pieces were treated immediately after cutting, by dipping in solutions or by dusting the pesticides on. After the treatment had dried, the seed pieces were placed in the open furrows and were inoculated by spraying them with a virulent 36-hour-old peptone-beef-broth culture of E. atroseptica, prepared as a water suspension in which two tubes (10 cc each) were used to 1 gallon of water. This method of inoculating the seed pieces also infested the soil surrounding them. The seed pieces were covered with soil immediately after inoculation was accomplished.

The plots were planted on May 5 and stand counts were recorded on June 10 each year. Yield data were taken on September 30 both years. The plots were replicated six times in randomized block.

In 1956 the fungicides and streptomycin, and the mixtures listed in Table 1, were tested. In 1957 the fungicides, streptomycin, and dieldrin, and the mixtures listed in Table 3, were tested. Since dieldrin is recommended for seed piece treatment in Pennsylvania, it was desirable to know if it would influence the efficacy of the other chemicals. Inoculated untreated seed pieces were planted as checks.

Laboratory Tests: In the laboratory the tests were conducted with Kennebec seed pieces. The potatoes were cut, treated, dried and inoculated by spraying with a virulent culture of E. atroseptica. The chemicals and mixtures used are listed in Table 2. The treated and inoculated seed pieces were placed in polyethylene bags and held for 1 week at room temperature before reading. The test was repeated three times and in each test the treatments were replicated three times. Phytotoxicity tests were made on uninoculated seed pieces.

All data were subjected to an analysis of variance and the resulting significant means were separated by Duncan's Multiple Range Test as outlined by Le Clerg (5).

RESULTS

Field Tests 1956: In 1956 37 treatments were used. The treatments and the resulting stands and yields are presented in Table 1. In this test, treatment with glyodin 1 1/2 quarts or ferbam 10 pounds per 100 gallons of water resulted in stands significantly greater than any of the other treatments, including streptomycin sulfate at 100 ppm. In no case did a mixture of streptomycin and a fungicide result in a stand significantly greater than where the fungicide was used alone. In some instances treatment with mixtures resulted in stands statistically significantly lower than where the fungicides were used alone; this was so when glyodin, ferbam (Fermate), captan, and dichlone (Phygon) were used.

There was an average of 1.9 sprouts per plant in the entire experimental field in 1956, 1.8 in untreated checks, and 2.1 in plots where streptomycin was used alone. The differences were not statistically significant.

Although significant differences were obtained in stand due to the treatments, no such differences were apparent in the subsequent yields (Table 1).

[1] Contribution No. 234 from the Department of Botany and Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication May 27, 1958 as paper No. 2259 in the Journal Series.

FIGURE 1. A: Left -- untreated, uninoculated. Right -- phytoxicity due to treatment with 10 pounds Fermate-100 gallons. B: Left -- treated with captan 2 pounds-100 gallons. Right -- phytoxicity due to treatment with 10 pounds Phygon-100 gallons.

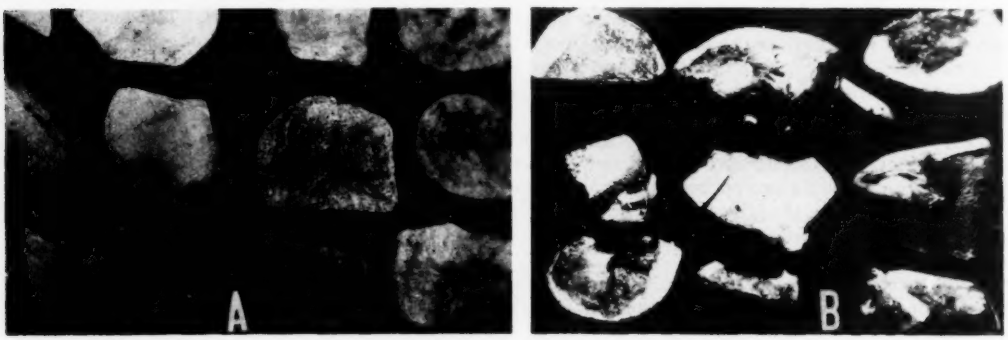


FIGURE 2. A: Left -- untreated uninoculated. Right -- uninoculated but treated with Spergon 2 pounds-100 gallons. B: Untreated but inoculated with E. atroseptica in laboratory studies.

Laboratory Tests: The laboratory tests conducted in the winter 1956-57 as a prelude to the field tests of 1957 indicated that streptomycin at 100 ppm will control seed piece rot due to E. atroseptica when used in the laboratory and in the absence of soil. The results are summarized in Table 2. In these tests mixtures of zineb (Dithane Z-78), captan, Fermate 5 pounds, griseofulvin, chloranil (Spergon), and streptomycin were statistically better control agents for seed rot than was streptomycin alone. All treatments except Phygon at 5 pounds per 100 gallons and dieldrin at 3 pounds were better than the untreated check. Although not always statistically evident, mixtures of fungicides, streptomycin and dieldrin always gave better control than the fungicides alone or the fungicides with streptomycin. Phytotoxicity was noted when Fermate 10 pounds or Phygon 10 pounds per 100 gallons was used. Actual disintegration and collapse of tissue occurred. (Figures 1 and 2)

Field Tests, 1957: The same treatments tested in the laboratory were evaluated in the field in 1957. Statistically significant decreases in stand were noted when the treatments were compared with the non-treated check. Though results were not different statistically it should be noted that, with the exception of griseofulvin, when the fungicides were used alone stands were always larger than where fungicides were mixed with streptomycin (Table 3). When dieldrin was added to the mixture this tendency to reduced stands was reversed. Laboratory

Table 1. Stand of plants from potato seed pieces treated with various chemicals. Field 1956.

Treatment	Rate 100 gal.	Stand Percent	Statistical Significance (5%)	Yield lbs.
1. Glyodin	1½ qts.	100		15.8
2. Fermate	10 lbs.	95		22.1
3. Catechol	100 p.p.m.	92		19.7
4. Catechol + Streptomycin	100 p.p.m. + 100 "	90		22.8
5. Spergon + Streptomycin	5 lb. + 100 p.p.m.	89		19.6
6. Vancide A	dust*	88		17.9
7. Nabam (D14) + Streptomycin	2 qts. + 100 p.p.m.	88		19.6
8. Captan	4 lbs.	86		17.6
9. Spergon	5 lbs.	86		16.5
10. Phygon	10 lbs.	85		19.5
11. Streptomycin	100 p.p.m.	85		21.1
12. Vancide M	dust	84		19.9
13. Vancide I	dust	84		16.1
14. A.C. 8599	15 lbs.	84		18.6
15. Griseofulvin	100 p.p.m.	84		24.1
16. Thioneb	4 lbs.	84		14.4
17. Glyodin + Streptomycin	1½ qt. + 100 p.p.m.	84		16.0
18. Vancide Z	dust	82		20.2
19. Terramycin + Streptomycin	100 p.p.m. + 100 p.p.m.	82		16.5
20. Nabam (D14)	2 qts.	81		16.5
21. A.C. 14307	15 lbs.	81		17.0
22. Terramycin	100 p.p.m.	81		21.6
23. Glyodin + $MnSO_4$	1½ qts. + 141 gms.	80		18.7
24. Neomycin	100 p.p.m.	80		8.0
25. Dithane Z-78	2 lbs.	80		17.0
26. No treatment		80		18.5
27. Captan + Streptomycin	4 lbs. + 100 p.p.m.	80		21.1
28. Thioneb + Streptomycin	4 lbs. + 100 p.p.m.	79		16.9
29. Dyrene	4 lbs.	79		21.0
30. Dithane Z-78 + Streptomycin	2 lbs. + 100 p.p.m.	79		19.9
31. Stanobrite	100 p.p.m.	78		18.7
32. Omadine Sulfate	100 p.p.m. + 100 p.p.m.	76		15.9
33. Dyrene + Streptomycin	4 lbs. + 100 p.p.m.	76		16.9
34. Fermate Streptomycin	10 lbs. + 100 p.p.m.	75		15.5
35. Stanabrite	50 p.p.m.	74		11.6
36. Phygon + Streptomycin		68		11.4
37. CA272		58		12.9
			F = 1.716	

Table 2. Control of potato seed rot caused by E. atroseptica. Laboratory.

Treatment*	Percent seed rot	Statistical Significance 5%
1. Z-78 2 lb. + Strep.	2.6	
2. Captan 2 lbs. + Strep. + Dieldrin	2.6	
3. Captan 2 lb. + Strep.	2.6	
4. Fermate 5 lb. + Strep. + Dieldrin	4.1	
5. Griseofulvin 100 p.p.m. + Strep. + Dieldrin	4.1	
6. Griseofulvin 100 p.p.m. + Strep.	4.1	
7. Fermate 5 lb. + Strep.	6.6	
8. Spergon 2 lb. + Strep.	8.0	
9. Spergon 2 lb. + Strep. + Dieldrin	13.3	
10. Fermate 10 lb. + Strep. + Dieldrin	16.0	
11. Streptomycin 100 p.p.m.	16.0	
12. Z-78 2 lb. + Strep. + Dieldrin	17.3	
13. Griseofulvin 100 p.p.m.	21.3	
14. Glyodin 1½ qt. + Strep. + Dieldrin	22.6	
15. Strep. + Dieldrin	22.6	
16. Phygon 5 lb. + Strept. + Dieldrin	25.3	
17. Fermate 10 lb. + Strep.	29.3	
18. Phygon 10 lb. + Strep. + Dieldrin	29.6	
19. Phygon 10 lb. + Strep.	33.3	
20. Phygon 5 lb. + Strep.	33.6	
21. D-14 1½ qt.	36.0	
22. Captan 2 lb.	40.0	
23. Glyodin 1¼ qts. + Strep.	44.0	
24. D-14 1½ qts. + Strep. Dieldrin	48.0	
25. Spergon 2 lb.	49.3	
26. Phygon 10 lb.	53.3	
27. Fermate 5 lb.	56.0	
28. Glyodin 1½ qt.	56.0	
29. Fermate 10 lbs.	57.3	
30. D-14 1½ qts.	58.6	
31. Z-78 2 lb.	65.3	
32. Dieldrin 3 lb.	72.0	
33. None	81.3	
34. Phygon 5 lb.	84.0	F = 4.22

* Streptomycin as streptomycin sulfate at 100 p. p. m. Dieldrin at 3 lbs. per 100 gallons of water and fungicides at specified rates in 100 gallons water.

Table 3. Stand of plants from potato seed pieces treated with various chemicals. Field 1957.

Treatment*	Percent Stand	Statistical Significance 5%
1. Phygon 10 lb.	73	
2. Z-78 1½ qts.	72	
3. Phygon 10 lbs. + Strep. + Dieldrin	70	
4. Phygon 5 lb.	68	
5. Phygon 10 lbs. + Strep.	68	
6. Z-78 2 lb. + Strep. + Dieldrin	68	
7. Phygon 5 lb. + Strep. + Dieldrin	67	
8. Spergon 2 lb.	65	
9. Captan 2 lb.	65	
10. Captan 2 lb. + Strep. + Dieldrin	64	
11. Glyodin 1¼ qt.	63	
12. Griseofulvin 100 p.p.m. + Strep.	63	
13. Fermate 5 lb.	62	
14. Dieldrin 3 lb.	61	
15. Captan 2 lb. + Strep.	60	
16. Griseofulvin 100 p.p.m. + Strep.	60	
17. Fermate 10 lb. + Strep. + Dieldrin	60	
18. Streptomycin 100 p.p.m.	59	
19. Phygon 5 lb. + Strep.	58	
20. Fermate 10 lb.	57	
21. Fermate 5 lb. + Strep. + Dieldrin	57	
22. No treatment	55	
23. Streptomycin + Dieldrin	52	
24. D-14 1½ qts.	51	
25. Spergon 2 lb. + Strep. + Dieldrin	50	
26. Griseofulvin 100 p.p.m.	49	
27. Z-78 2 lb. + Strep.	48	
28. Glyodin 1¼ qt. + Strep.	47	
29. Fermate 5 lb. + Strep.	47	
30. Fermate 10 lb. + Strep.	45	
31. Glyodin 1¼ qt. + Strep. + Dieldrin	42	
32. D-14 1½ qt. + Strep.	41	
33. Spergon 2 lb. + Strep.	41	
34. D-14 1½ qt. + Strep. + Dieldrin	26	F = 1.750

* Streptomycin as Streptomycin Sulfate at 100 p. p. m. Dieldrin at 3 lbs. and fungicides at rates specified per 100 gallons of water.

Table 4. Yield of Katahdin potatoes from seed pieces treated with fungicides, Streptomycin, Dieldrin and their mixtures 1957.

Treatment*	Yield Pounds	Statistical Significance 5%
1. Glyodin 1¼ qt.	105.6	
2. Spergon 2 lb.	100.0	
3. Phygon 10 lb.	99.7	
4. Phygon 5 lb.	98.7	
5. Fermate 5 lb.	96.6	
6. Fermate 10 lb.	96.2	
7. Captan 2 lb.	95.8	
8. Phygon 10 lb. + Strep. + Dieldrin	94.1	
9. Phygon 10 lb. + Strep.	93.7	
10. Z-78 2 lb.	92.6	
11. Phygon 5 lb. + Strep. + Dieldrin	86.3	
12. Captan 2 lb. + Strep. + Dieldrin	82.9	
13. Griseofulvin 100 p.p.m. + Dieldrin	80.8	
14. Streptomycin	78.4	
15. Fermate 5 lb. + Strep. + Dieldrin	78.4	
16. D-14 1½ qts.	78.1	
17. Griseofulvin 100 p.p.m. + Strep.	78.0	
18. Captan 2 lb. + Strep.	77.9	
19. No treatment	76.5	
20. Fermate 10 lbs. + Strep. + Dieldrin	76.1	
21. Z-78, 2 lb. + Strep. + Dieldrin	76.0	
22. Phygon 5 lb. + Strep.	75.6	
23. Griseofulvin 100 p.p.m.	74.7	
24. Glyodin 1¼ qt. + Strep. + Dieldrin	73.7	
25. Streptomycin + Dieldrin	71.5	
26. Dieldrin	70.5	
27. D-14 1½ qt. + Strep.	67.7	
28. Spergon 2 lb. + Strep. + Dieldrin	64.0	
29. Fermate 10 lb. + Strep.	55.5	
30. Spergon 2 lb. + Strep.	54.9	
31. Glyodin 1¼ qt. + Strep.	52.7	
32. Fermate 5 lb. + Strep.	51.7	
33. Z-78 + Strep.	48.0	
34. D-14, 1½ lb. + Strep. + Dieldrin	43.8	F = 1.7985

* Streptomycin as Streptomycin Sulfate at 100 p. p. m., Dieldrin at 3 lb. and fungicides at rates specified in 100 gallons water.

tests demonstrated that dieldrin possesses no bactericidal value (Table 2). Although statistically significant differences in stand occurred between treatments in 1957, and also statistically significant differences in yield, no increase or decrease due to treatments was found when the mean of the untreated check was compared with the other means. Real decreases in stand occurred when streptomycin was added to glyodin, Fermate 10 pounds, Fermate 5 pounds, or Spergon 3 pounds (Table 4).

DISCUSSION AND CONCLUSIONS

Reasonable conclusions can be drawn from the data presented: A -- Seed piece rot due to Erwinia atroseptica can be controlled in the laboratory in the absence of soil with streptomycin sulfate at 100 ppm. B -- Mixtures of the fungicides and dieldrin with streptomycin did not have an adverse effect on control in the laboratory. C -- No correlation exists between the results of field testing and of laboratory testing of potato seed treatments. D -- None of the treatments tested gave commercial control of seed piece rot due to E. atroseptica in the field. It is recognized that once a treated seed piece is placed in soil many factors are introduced into the experiment that are beyond the control of the experimenter. The streptomycin may have been adsorbed onto the soil particles and was therefore not available for the control of the bacteria. Other fungi or bacteria may have influenced the pathogenic action of the inoculum, or may have rotted the seed piece in the presence of the streptomycin. E -- Because lower stands and yields occurred in these plots where fungicide and streptomycin mixtures were used, it seems reasonable to conclude that treatment with these mixtures might result in lower stands and yields than no treatment in commercial potato growing ventures. F -- A third chemical added to the fungicide-streptomycin mixture may influence the action of the mixture. This is evident where dieldrin was used in these tests. It appears as if dieldrin may reduce phytotoxicity of the mixtures.

Literature Cited:

1. BONDE, REINER. 1953. Preliminary studies on the control of bacterial decay of the potato with antibiotics. Am. Potato J. 30: 143-147.
2. BONDE, REINER. 1955. Antibiotic treatment of seed potatoes in relation to seed-piece decay, black leg, plant growth and yield-rate. Plant Disease Reptr. 39: 120-123.
3. BONDE, REINER, and PAULO de SOUZA. 1954. Studies on the control of potato bacterial seed-piece decay and black leg with antibiotics. Am. Potato J. 31: 311-316.
4. FINK, H. C., O. D. BURKE, R. S. KIRBY, and L. P. NICHOLS. 1956. Potato seed piece treatments in Pennsylvania. Plant Disease Reptr. 40: 125.
5. Le CLERG, E. L. 1957. Mean separation by the functional analyses of variance and multiple comparisons. U. S. Dept. Agr., ARS 20-3.
6. PALM, E. T., and R. A. YOUNG. 1957. The compatibility of certain organic fungicides and antibiotics in treatment mixtures as indicated by stability and phytotoxicity. Plant Disease Reptr. 41: 151-155.
7. WAGGONER, PAUL E. 1956. Chemical treatment of potato seed in Connecticut 1955. Plant Disease Reptr. 40: 411-413.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, THE PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNSYLVANIA

"TORBÓ", A TROPICAL DISEASE OF POTATOES[1]

Ricardo A. Rodríguez[2]

INTRODUCTION

"Torbó" is the name applied to a serious disease of potatoes in Costa Rica. Although the disease has recently been described for the first time in a technical bulletin (2), it has been recognized by potato farmers for a long time.

Based on symptomatology and etiological studies, and from observations on disease development, there is no report in the literature which describes this malady. Certain similarities between "charcoal rot", caused by Macrophomina phaseoli (Maubl.) Ashby, as described by Bazán in Perú (1), and "torbó", could lead to confusion of the two diseases.

"Torbó" is usually found in level or moderately sloping fields, where water tends to accumulate. When the disease appears in the field, it attacks plants in a scattered manner throughout the infected area, or in patches, showing similarity in its distribution to "black canker" of coffee, caused by Rosellinia bunodes Berk. & Br.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The disease is widely distributed in the potato fields of Costa Rica. Although no economic study to determine the losses caused by it has yet been made, nevertheless farmers consider "torbó", when established in the field, to be second only to late blight (Phytophthora infestans (Mont.) DeBary) in seriousness. Tubers lose commercial value; loss estimates range from 10 to more than 50 percent of the crop in the soil, with subsequent rot in storage. In most cases tubers rot completely before harvest time (See Fig. 1).

ENVIRONMENTAL CONDITIONS

As already noted, the disease becomes more serious in soils where water accumulates, due to their physical condition or poor drainage. This statement is supported by field observations made in a dry period, in which tubers located deep in the furrow, where moisture content was higher and more constant, gave evidence of more damage.

Although "torbó" has been found in the Irazú highlands[3] at more than 2500 meters altitude, it occurs much more frequently at lower levels, in warmer soils of about 1500 meters of altitude, where the potato fields of Pacayas and Cot are located. More exact relations of temperature to the growth of the organism which causes "torbó", will be determined in the laboratory at a later date when proper facilities are available.

SYMPTOMS

Subterranean parts of the plant (as a rule, not all of the tubers in a hill become diseased), in the initial phase of the disease appear covered by a white mat of a fan-like, fast-growing mycelium. Such a condition is known by the farmers as "white torbó" (Fig. 2).

In potatoes, as in other host plants, "torbó" may cause stem canker and root rot, which results in wilting of the young plants. Because of this consideration, it is possible that part of what the farmers in Costa Rica call "bacterial wilt" (Pseudomonas solanacearum E. F. Sm.)[4] is in fact caused by "torbó" infection.

Because of the fact that "white torbó" is a superficial growth, the damage can be checked when the tubers are exposed to aeration and dryness. Nevertheless, the fungus will resume growth in storage, when moisture conditions are higher and become favorable for the pathogen.

---

[1] There is no meaning for the word "torbó", except that it is widely used by potato farmers.

[2] Plant Pathologist Research Assistant to Plant Pathology Division, M. A. I., San José, Costa Rica.

The author wishes to acknowledge valuable help in laboratory and field experiments of Ings. Carlos A. Soto and Carlos L. Bianchini of this office.

[3] On the slopes of the Irazú Volcano, in the northern part of the province of Cartago, Costa Rica, most of the potato fields are planted.

[4] Bacterial wilt is called "Maya" in Costa Rica.

The fungus attacks tubers as they start to form and from then on, until they reach the ripening stage.

Afterwards, under the white fungus mat, a dark crust is formed, with a rugose, netted surface (Fig. 3), from which radial necrotic streaks invade the flesh. The symptoms may completely surround the tuber, and when the disease is advanced, a cross section will show a cortical band of necrotic streaks which seldom reach the pith. Such tubers usually become hollow because of rotting due to secondary organisms (Fig. 4).

This advanced stage of the disease is called "black torbó" and has been found in other vegetables and weeds.

From inoculation experiments, it has been determined that it takes approximately 5 to 6 days to progress from the white to the black stage of the necrotic streaks.

From fresh tubers collected in a badly infested field, by longitudinal and cross sections, the following observations were made in the laboratory:

a) Necrotic streaks penetrate evenly all around the tuber in such a way as to form a characteristic circular band.

b) In a young tuber, streaks appear to run lengthwise from the point of infection, and eventually as the tuber grows the necrosis will change direction, becoming radial at the end.

c) Streaks will form regardless of the kind of tissue, but usually fail to appear in the medullary region, on account of putrefaction produced by secondary organisms.

d) Slices from diseased specimens set in Petri dishes at room temperature produced abundant growth of a fungus starting from the lesions. By microscopic examination this fungus was identified as the one in the white stage of the disease. Therefore, the necrotic streaks are caused by rhizomorphs of the fungus, penetrating the tuber at right angles to the surface.

The disease also occurs in other weed and vegetable hosts. Although no host range studies have been made, both white and black stages of "torbó" have been found in cauliflower, beet, and carrot. (Figs. 5, 6). It has also been found in such weeds, commonly found in potato fields, as "nabillo" (Brassica campestris) and "bledo blanco" (Amaranthus dubius)[5]

STUDIES ON THE ETIOLOGY OF THE DISEASE

When experiments for the identification of the causal organism of this disease were started, the white fungus that covers the skin of tubers was isolated. Unfortunately, the first isolate made proved to be non-pathogenic. Many other fungi and bacteria were isolated and several methods of inoculation were tried. Neither infection nor reproduction of characteristic symptoms of the disease was obtained until a second strain of the white fungus, which differed in growth and pigmentation from the first strain, was isolated. This second isolate will be called from now on Isolate A.

Materials and Methods

In the inoculation experiments, a fruit jar (3 inches in diameter) was used as a moist chamber, covered with absorbent paper adjusted with a rubber band as a lid.

Fresh immature tubers were collected for the experiment (experience shows that immature tubers are infected more rapidly than mature ones). Tubers were immersed in 10% clorox (commercial 5.25% sodium hypochlorite) for 30 seconds, for superficial disinfection.

All potatoes were slightly scratched with sterile scalpels to produce wounds; then they were wrapped in young potato-dextrose agar cultures of the A isolate. Some tubers were left unwrapped in a separate jar for the control.

Inoculated and non-inoculated tubers were then buried in moist sterile vermiculite (mica) which was pressed against the tubers in order to assure good contact with the inoculum. Its water-absorbent properties, as well as its physical condition, were apparently favorable for the growth of the fungus, which 2 days later showed a white mycelium coming out through the

[5]Wild hosts were identified by Prof. José Ma. Orozco, Botanical Assessor to M. A. I., San José, Costa Rica.

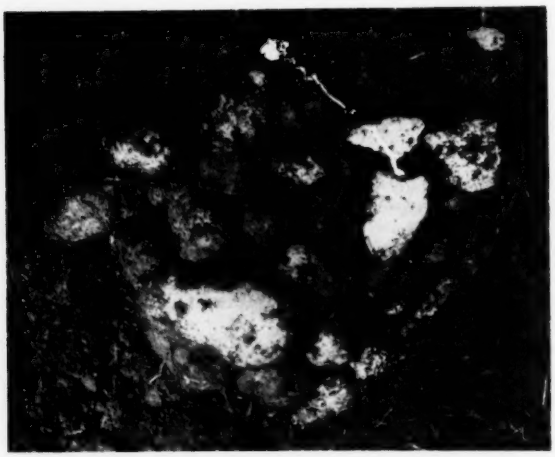


FIGURE 1. Infectious site of "torbó". In severe cases all the tubers in the hill may become diseased.

FIGURE 2. "White torbó". Initial stage of the disease when the tuber appears covered with a dense white coat of fungus growth.

FIGURE 3. Transitional stage from the white to the black phase of the disease. Note the dark, rugose crust of the tubers which will later become a black and hardened skin.

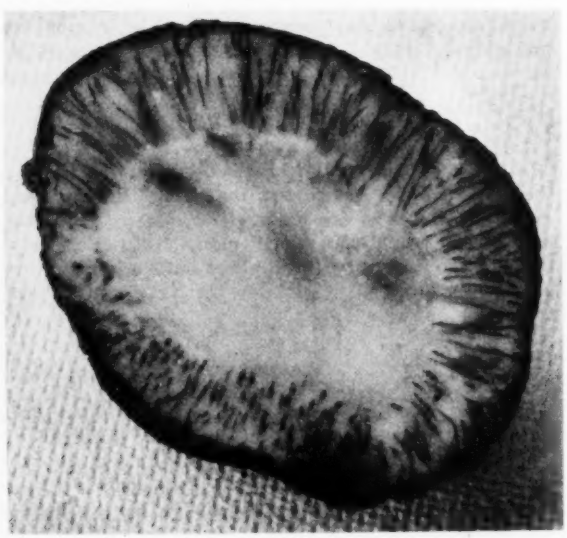


FIGURE 4. Cross section of a tuber showing the advanced stage of the disease ("black torbó"), with characteristic radial necrotic streaks.

FIGURE 5. White stage of "torbó" in beet. It causes wilting of this plant, as well as of potato and carrot.

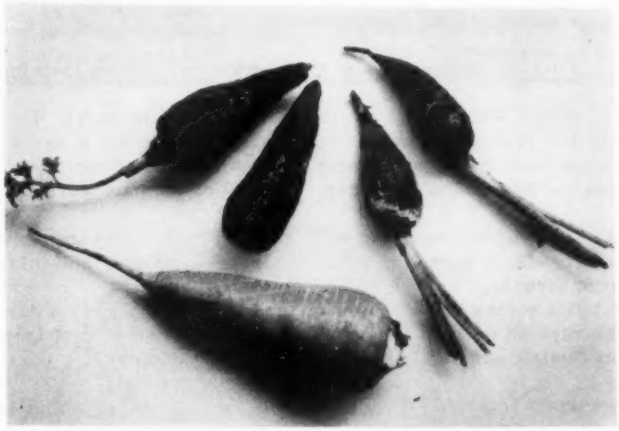


FIGURE 6. Carrot roots severely affected by "torbó" infection. Note dark aspect and decrease in size as compared with healthy root below.

vermiculite. Six days after inoculation the white stage of the disease was obtained on the tubers; the fungus formed typical rhizomorphs in the inoculating chamber (Fig. 7). In 2 more weeks, the characteristic necrotic streaks were noted in cross sections (Fig. 8).

After reisolation of the fungus from the lesions produced by inoculation, and verification of its cultural characteristics, the Koch postulates were fulfilled, and therefore the pathogenicity of the organism demonstrated.

Cultural Characteristics of the Fungus on PDA

"A" Isolate: Initial growth is rapid, with long erect mycelium, as in Rhizoctonia; then growth slows down forming a very irregular colony of a white flat mycelium except for some vigorous aerial marginal growths. In the center, or scattered through the colony, masses of mycelium which form sclerotia-like bodies collect. At first light in color, these later turn dark-brown. Old sclerotia are almost black, conspicuous, usually with dark watery exudations.

Mycelium is septate with long cells, wider at the base of each cell. Originally hyaline or light brown, the mycelium becomes darker with age.

As the culture ages, cells become shorter and show more branching.

The fungus has not been found to sporulate, either in culture or in nature.

Colony and morphologic characteristics show that this fungus belongs to the genus Rosellinia (Fig. 9). Rosellinia radiciperda Mass. has been reported as being able to attack potatoes (3, page 164). "Torbó" has been observed on potatoes on a soil badly infested with Rosellinia bunodes Berk. & Br., near a coffee plantation in the Italian Colony, in the southern part of Costa Rica.

"B" Isolate: Starts with a very white, cottony mycelium of a short spread, which turns dark with age. It produces a low-growing, round colony with irregular borders. Other cultural features are the same as isolate A. Non-virulent.

Test for Differences in Pathogenicity Between Isolates

Three series of potato slices were set in Petri dishes (two each). One series was inoculated with Isolate A, the second series was inoculated with Isolate B and a third series was left uninoculated for the control. The experiment was maintained at room temperature. The slices were examined from the fifth to the tenth day. The following data were obtained:

a) The series inoculated with Isolate A (virulent) showed abundant fungus growth, a dark superficial crust on the slice, and internal necrotic streaks.

b) The series inoculated with isolate B showed only a dark crust and depression on the flat side of the slice at the place of inoculation.

c) Control slices showed no signs of the disease.

The above experiment indicated the existence of two biotypes. Other biological features of this study will be carried out as laboratory facilities become available.

DISEASE CYCLE

The fungus lives in the soil in crop refuse where it forms sclerotia. Young potato plants which become infected may show their lower stems and underground parts covered with a dense coat of a white mycelium. Under this white coat, cankers develop, which result in wilting and invasion by secondary organisms which completely rot the infected tissues.

In adult plants, the disease may appear at almost any stage of development of the tubers. Healthy tubers that remain in the soil for more than a month after the vegetative period has ended, under high moisture conditions, may become infected and disintegrate into a mucilaginous slime. Thus it can be assumed that the longer the crop stays unharvested, the greater will be the risk of infection.

There will be a large amount of inoculum in the crop residues for the new planting, usually resulting in a higher degree of infection, if no sanitary measures are taken.

FIGURE 7. Inoculation flask showing the growth of the fungus by rhizomorphs. Note a white coat of mycelium on top of the flask.

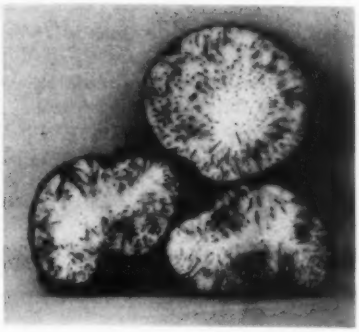


FIGURE 8. Cross section of diseased tubers inoculated with "A isolate." Streaks were produced throughout since no putrefaction occurred.

FIGURE 9. Hyphae of "Isolate A" (probably Rosellinia sp.) showing characteristic swellings of mycelium at the base of each cell (X about 225).

FIELD EXPERIMENTS ON CONTROL

Early in 1956, before the identity of the pathogen was established, some field experiments were undertaken with soil fungicides. Because of the small quantities of the chemicals applied in the first experiment, no satisfactory control of the disease was attained; nevertheless, there was some indication as to the effectiveness of some of them. A second test included those products which appeared to be most promising in the first test, and also other soil fungicides that became available at the time of the experiment (2).

Materials and Methods

In a highly infested field, plots of 12 square meters were used for treatment, each treatment being replicated three times in a randomized block design. The fungicides, except N-521, were applied twice with a hand sprinkler, with a 4-week interval, and the potato seed planted 3 weeks after the last fungicide application. This second treatment was intended to kill resistant forms of the fungus that were vegetatively inactive at the time of the first application.

One gallon of the fungicidal preparation was applied to each square meter. The moistened soil was then cultivated to a depth of approximately 15 cm to allow a good distribution of the fungicide. Following each fungicidal treatment, water was sprayed over the plots in order to obtain a good seal.

Volunteer plants were uprooted, and sanitation and severe cultivation was practiced in the field until the plants were well established and covered the ground.

The chemicals were applied at the following concentrations:

Stauffer N-521 Tetrahydro 3, 5, dimethyl 2 H-1, 3, 5-thiadiazine 2 thione	200 Kg/Ha
Vapam (Stauffer N-869) Sodium methyldithiocarbamate	75 Ltr/Ha
Terraclor 75% WP Pentachloronitrobenzene	250 Kg/Ha
Agallol (Bayer) Mercury methoxyethylchloride	250 Kg/Ha

Seed pieces were disinfected with Agri-Mycin 100 (Pfizer antibiotic containing 15% streptomycin plus 1.5% Terramycin) at 100 ppm, by 20 minutes immersion.

At harvest, the healthy and diseased tubers were weighed separately, and the results statistically analyzed from the percentage of infection for each one of the replications. Table 1 shows the results of the experiment.

Table 1. Differences in "torbó" infection as a result of fungicide application to the soil (dry season).

Treatment	Fungicide to the square meter	Total of three replicates
Stauffer N-521	20 grs/gal. 2	11.45
Vapam	7.5 ml/gal. 2	2.75
P.C.N.B.	25 grs/gal. 2	2.84
Agallol	25 grs/gal. 2	14.26
Check	--- ---	39.74
L.S.D. at 5% level		22.51
L.S.D. at 1% level		33.89

It may be seen that under the dry season conditions during which this experiment was conducted (see Table 2), all fungicides significantly reduced infection. Both Vapam and P.C.N.B. were significantly different from the check at the 1% level, although there was no statistical difference between treatments.

By increasing the amount of fungicide over that used in the preliminary experiment, and apparently, by treating the soil twice with a 3-weeks interval, positive results were obtained.

Table 2. Rainfall in relation to experiments with soil fungicides for the control of Rosellinia sp.[a] on potato.

Dry season Month	Precipitation (mm)	Wet season Month	Precipitation (mm)
November 1956	135.4	May 1957	224.4
December	450.7	June	158.5
January 1957	253.00	July	280.7
February	54.4	August	203.3
March	22.7	September	263.8
April	21.00	October	417.1
Total:	937.20	Total:	1547.8

Difference in precipitation between dry and wet season experiments: 610.6 mm

[a]Data obtained from the Servicio Meteorológico Nacional, Ministerio de Agricultura e Industrias, San José, Costa Rica.

Since potatoes are planted in Costa Rica the year round, another field experiment was planned for the next rainy season. During this very humid period (see Table 2), disease incidence is higher and the above method was tested again.

In this second experiment, seed was disinfected with Agri-Mycin 100 at 200 ppm.

Twelve square meter plots were used per treatment. Each treatment was replicated three times in randomized blocks, and the fungicides were applied in the same way as in the former experiment.

N-521 was not available for this experiment; instead two other chemicals were added[6] The fungicides were used at the following concentrations:

75% Pentachloronitrobenzene	250 Kg/Ha
70% Ziram (Fuclasin)	250 Kg/Ha
10% Phenyl Mercury Acetate (T.A.G.)	100 Ltrs/Ha
Sodium methyldithiocarbamate	100 Ltrs/Ha

When the potato plants were fully grown there was a heavy infestation of the potato tuber worm (Gnorimoschema operculella (Zeller)[7], and because of its damage to the tubers, bacterial wilt occurred (Pseudomonas solanacearum E.F. Smith), lowering the yield. However, evaluation of incidence of "torbo" was not interfered with, since the two diseases are easily separated.

Evaluation of results was done by weighing "torbó"-infected tubers in comparison with healthy and bacterial diseased tubers.

Although, as it may be observed from Table 3, the difference in percentage of infection from the best treatment as compared with the check was about equal to that of the former experiment, the analysis of variance indicated no statistical significance. This is explained by the fact that there was an extreme variation of the infection rate between replicates of the same treatment, thus giving a very large standard error.

[6]Acknowledgment is given to the following companies for supplying the chemicals used in the experiments: Stauffer Chemical Corporation; Chas. Pfizer & Co., Inc.; Bayer, Germany; Olin Mathieson Chemical Corp.; and Schering from Germany.

[7]The potato tuber worm was identified by Ing. Alvaro Cordero, Head of the Entomology Division, M.A.I., San José, C. R.

Table 3. Result of fungicide application to soil against Rosellinia sp. (rainy season).

Treatment	Fungicide to the square meter	Total of three replicates
Vapam	10 ml/gal. 2	23.67
P.C.N.B.	25 grs/gal. 2	25.95
Fuclasin	25 grs/gal. 2	43.70
T.A.G.	10 ml/gal. 2	51.02
Check	-----	66.82

From the results shown in Table 3, it can be concluded that unless a more effective method is developed for application during the rainy season, disease incidence will not economically be reduced with the fungicides here tested.

Having already identified the causal organism, bio-analytical assays will be undertaken towards a fungicide screening test in the laboratory. Other field test experiments are planned to try new methods of application and more accurate techniques for evaluation of results.

SUMMARY

The disease known as "torbó" is widely distributed throughout the potato fields of Costa Rica.

Where established, farmers consider "torbó" as second only to late blight in importance. Diseased tubers lose commercial value, with crop losses up to 50 percent or more.

Both the white and black stages of the disease are caused by a fungus that probably belongs to the genus Rosellinia, as demonstrated by inoculation experiments.

There seems to be more than one strain of the fungus.

Satisfactory control of the disease was attained in field experiments with the application of soil fungicides and sanitary practices.

Terraclor and Vapam were effective as drenches during dry-season conditions, in two applications at 3-weeks interval with 250 kilograms and 100 liters per hectare, respectively.

Literature Cited

1. BAZÁN de SEGURA, CONSUELO. 1956. Podredumbre Carbonosa de la papa. Estación Agrícola Experimental de la Molina, Ministerio de Agricultura, Lima, Perú, Boletín No. 63, March.
2. RODRÍGUEZ, R. A., CARLOS A. SOTO, and CARLOS L. BIANCHINI. 1957. El "torbó" de las papas. Ministerio de Agricultura e Industrias, San José, Costa Rica, Boletín Técnico No. 19.
3. STEVENS, F. L. 1925. Plant Disease Fungi. The Mac Millan Company, New York.

SECCION DE FITOPATOLOGIA, MINISTERIO DE AGRICULTURA E INDUSTRIAS, SAN JOSÉ, COSTA RICA

BOTRYTIS IN PEPPER SEEDBEDS AND ON YOUNG PLANTS IN THE FIELD

N. N. Winstead, J. C. Wells, and W. W. Reid

During 1957 and 1958 Botrytis blight was observed in several pepper (Capsicum frutescens L.) plant beds in southeastern North Carolina. In most of the cases where Botrytis blight was severe plants were growing under plastic sashes or in plastic greenhouses with inadequate ventilation. However, in a few instances the disease was severe under glass sashes. In all cases, the disease was associated with excessive moisture and high humidity. In severely affected beds, 75 percent or more of the plants were lost.

This disease was also observed in three fields in 1957 and 1958. In each case there was extensive necrosis on the stems of affected plants. Losses of stand ranged from 25 to 60 percent. Although none of the seed beds concerned were examined, it was suspected that infected plants were transplanted to the field.

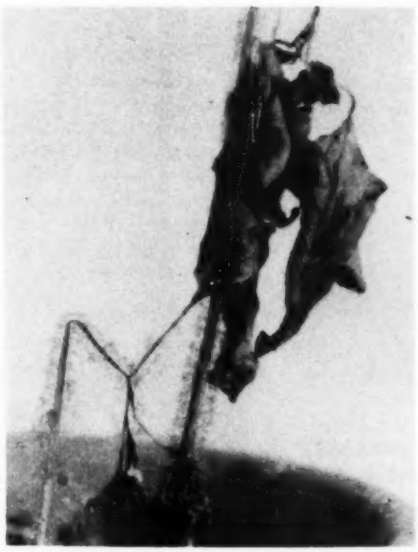


FIGURE 1. California Wonder pepper plants affected with Botrytis blight.

Inoculations were made with isolates of Botrytis obtained from tomato fruits, collected from a commercial greenhouse at Raleigh, North Carolina, and from diseased pepper seedlings, collected from a seedbed at Faison, North Carolina. California Wonder pepper seedlings and young plants with about 10 to 12 true leaves were inoculated with an aqueous spore suspension containing approximately 50,000 spores per ml. Inoculated plants were incubated at 65° F. Symptoms developed only on plants maintained under humid conditions. The first symptoms appeared 2 days after inoculation and consisted of a slight cupping of the two youngest leaves, particularly at the tip of the leaf. Leaves were usually chlorotic 3 days after inoculation and in most cases the young leaves had slight watersoaking and necrosis. Within 3 to 4 days watersoaked spots had developed on the older leaves. Infected areas on the leaves enlarged and the fungus appeared to grow down the leaf petioles into the main stem, and 6 days after inoculation the entire plant was usually affected. The isolates from tomato fruits and pepper seedlings were indistinguishable morphologically and pathogenically and were identified as B. cinerea Fr. Severely affected plants inoculated 6 days previously are shown in Figure 1.

Control of the disease was obtained in preliminary tests with a mixture of approximately 1 pound captan and 1 1/2 pounds pentachloronitrobenzene per 100 gallons of water. These rates are those of the active material. Treatments were applied as a drench at a rate of 1/2 gallon per square yard at 5-day intervals.

Botrytis cinerea Fr. has frequently been encountered in the seedbed on lettuce, Lactuca sativa L., seedlings and occasionally on lettuce plants in the field in North Carolina; this fungus has also been observed on greenhouse grown tomato, Lycopersicon esculentum Mill., fruits; however, no Botrytis blight of pepper seedlings and transplants has previously been reported in North Carolina.

PLANT PATHOLOGY, NORTH CAROLINA STATE COLLEGE, RALEIGH, NORTH CAROLINA AND COASTAL PLAIN VEGETABLE RESEARCH STATION, FAISON, NORTH CAROLINA

DISTAL-END ROT OF SWEETPOTATOES OF THE ACADIAN VARIETY

W. J. Martin

The Acadian variety of sweetpotato was released by the Louisiana Agricultural Experiment Station in 1956. It originated from a seedling grown for the first time in 1951. Since 1954 a disease peculiar to this variety has been observed. The disease is characterized by a decay restricted to the distal end of the root (Figure 1). Incidence of the disease has varied from none in a few lots to over 30 percent of the roots affected in others. The disease is not evident until after harvest, usually appearing while curing or in storage. The decay does not progress very rapidly and is always restricted to the distal end of the roots. Rarely is more than one-third of the root affected.

Numerous attempts to isolate a causal fungus or bacterium from the decaying tissue and surrounding tissues have not been successful. The name distal-end rot is suggested.

FIGURE 1. Distal-end rot of the Acadian sweetpotato.

DEPARTMENT OF PLANT PATHOLOGY, LOUISIANA AGRICULTURAL EXPERIMENT STATION, BATON ROUGE, LOUISIANA

UNUSUAL OCCURRENCE OF CERTAIN DISEASES IN LOUISIANA

J. B. Sinclair, N. L. Horn and E. C. Tims[1]

ASTER YELLOWS IN SHALLOT

During the past growing season, October 1957 to April 1958, aster yellows (virus) was much more prevalent than usual in shallot fields all over the Southern section of the State. The disease was especially conspicuous in the early plantings during October and November of 1957. The normal shallot plant is dark green in color, a shade of green quite different from that of most other plants in the fields. The bright yellow color of the aster yellows infected plants makes them very easy to distinguish from the healthy ones.

The disease often appears first on one side of the bunch and gradually spreads until the entire bunch is a bright yellow color. All the leaves on some bunches turn yellow at about the same time. Severely diseased shallots never form bulbs. In this way the disease is self eliminating to some extent. Aster yellows may be carried over in the sets produced on portions of the bunches that were infected late in the season.

Counts were made of infected plants in a number of shallot fields in the period from October 15 to December 15, 1957. Infected bunches ranged from a trace to 8 percent in certain areas. Diseased plants are a total loss to the grower because of their yellow color. The diseased plants are always left in the field when the crop is harvested and are usually plowed under within a short time.

PEPPER DISEASES

Phytophthora sp. was found in several bell pepper (Capsicum frutescens, horticultural variety unknown) plantings in the Ponchatoula area of Louisiana in the spring of 1958. Weather conditions were ideal for the development of the fungus. Both stems and leaves were affected. The leaf spots were water soaked in appearance with somewhat darker green borders, and measured up to 4 cm in diameter. Several larger spots side by side covered the entire lateral dimension of some leaves. Defoliation was severe over the entire plantings, in fact, only a few terminal leaves remained on many plants. On the stems the organism caused black lesions that sometimes nearly girdled the larger branches, the infection usually occurring at the crotch. Leaf sections were plated on potato-dextrose agar. Within a week many sporangia were found on the cultures isolated. The organism was identified as Phytophthora sp. and assumed to be P. capsici. Departmental files indicated that the latest previous report of this disease in Louisiana was in 1944.

Stemphylium sp. was found in several bell pepper beds in St. James Parish. The general distribution of the organism was not known. However, where the disease occurred the plants were severely infected and unfit for transplanting. The organism caused leaf spots up to 3 or 4 mm in diameter. The centers were tan to very light tan with dark red margins. Centers of older spots sometimes fell out. Defoliation was so severe that only a few terminal leaves remained on most of the plants. Severely infected plants were 3 to 5 inches tall, whereas those with few to no spots were about 6 to 9 inches tall with many leaves. Plants with similar spots were found scattered in most hot beds examined. Healthy plants became severely infected when inoculated with isolates of the fungus in question.

TIMBER ROT OF TOMATOES

Timber rot of tomatoes, incited by Sclerotinia sclerotiorum, was reported by Plakidas in 1957 as an unusual disease on mature field plants, at the Plaquemines Parish Experiment Station located in the extreme southeastern part of Louisiana[2]. The disease appeared again in cold frames, attacking tomato seedlings at the same location during March 1958. About 15 percent of the plants were infected and later killed by the fungus. Stem rot was observed a week later on tomato plants growing in a plastic greenhouse on the station. No diseased plants were found in the field during the early spring of 1958. In May 1958, however, the disease was found

---

[1] Assistant Plant Pathologist, Associate Plant Pathologist, and Plant Pathologist respectively, Louisiana State University Agricultural Experiment Station, Baton Rouge, Louisiana.

[2] Plakidas, A. G. 1957. New or unusual plant diseases in Louisiana. Plant Disease Reptr. 41:643.

on maturing field tomatoes in the northeastern part of the State. About a 5 percent loss was reported by the county agent. Infected plants had typical white, fluffy mycelial growth on stems and petioles. Large black sclerotia were found on mature greenhouse and field plants, but were produced on infected seedlings only after they were brought into the laboratory. Most severely infected plants wilted and died.

BUCKEYE ROT OF TOMATOES

A tomato disease survey was made in St. Bernard Parish on May 27 and 28, 1958. Buckeye rot of tomato fruit was found in each of 13 fields inspected. Estimated losses due to the disease varied from 10 to almost 50 percent. Phytophthora sp., Fusarium sp., and Alternaria sp., were found associated with the fruit lesions, the first named being most prevalent. Specimens of tomato fruit with buckeye rot symptoms were received from Lafourche Parish on June 9, 1958. All of the tomatoes in these areas were staked. The disease appeared as a target-like pattern, first on the fruit of the lower clusters and later, in some cases, on the second and third clusters. Foliage blight symptoms were absent, even in one case where the grower had used no fungicide for disease control. This grower had the greatest fruit loss due to the disease, about 50 percent of his crop. Growers who had followed the recommended spray schedule had less fruit infection.

WEATHER CONDITIONS

Weather conditions were an important factor in the occurrence of these diseases, especially those of pepper and tomato. Cool temperatures accompanied by frequent rains continued to be prevalent through May and into the first week in June.

LOUISIANA STATE UNIVERSITY AGRICULTURAL EXPERIMENT STATION, BATON ROUGE, LOUISIANA

A DIEBACK OF BELMONT GARDENIAS

Philip Brierley[1] and Paul Lorentz[1]

Summary

A dieback of Belmont gardenias in Pennsylvania greenhouses is described and attributed to root smothering resulting from over watering.

In spring of 1955 dieback of Gardenia augusta (L.) Merr. was brought to our attention by Pennsylvania growers. Three of four growers of the Belmont variety had this disorder in their greenhouses. All had been producing gardenias for many years, but had noted the dieback disease first during the preceding 4 or 5 years even though they had introduced no new stock.

Defoliation is the first symptom noted, as fallen leaves attract attention to the diseased plant. Bright-yellow veinbanding and brown necrotic lines sometimes appear before or after the leaves fall. In necrotic leisons, terminal or intercalary in young twigs, all tissues external to the xylem are collapsed. The necrotic areas extend slowly and erratically and often enclose islands of apparently normal tissue. Plants die when the main stem is girdled. Roots typically remain white and normal in appearance until late stages of dieback. Dieback was not evident during the summer, but appeared during the period from October to April in 1-, 2- or 3-year-old plants. Incidence was usually less than 1 percent, but reached 5 to 10 percent on one greenhouse bench. Affected plants appeared singly or in small groups, scattered at random in greenhouses.

No pathogens were found associated with dieback by pathologists at Pennsylvania State University or at Beltsville. Fertilizer practices were ruled out as possible causes by New Jersey Experiment Station specialists and fumigants by Floyd F. Smith of Entomology Research Division, Beltsville. We were asked to investigate dieback as a disease of possible virus origin.

Plants in early stages of dieback supplied us by George Gilmore of Willow Grove, Pennsylvania, in April 1955 died 9 to 11 weeks after being moved to Beltsville. Additional plants selected by Mr. Gilmore at the earliest recognizable leaf-drop stage in November 1955 also died 7 to 11 months after being moved. This finding shows that Mr. Gilmore was able to diagnose dieback in early stages with high accuracy. These plants served as source material in our transmission trials. Herman Lederer of Parker Ford, Pennsylvania, who has never had dieback, furnished four healthy Belmont gardenias. The Lederer plants and also a plant of the Veitchii variety long grown under glass at Beltsville were propagated and used as test plants. In our experimental culture all gardenias were grown in pots although commercially they are grown in benches or beds.

Sap inoculation and Yarwood's fresh leaf method of inoculation from dieback gardenia induced no effect in gardenia, tobacco, snapdragon, Gomphrena, or China aster. Needle-prick inoculation leaf to leaf from diseased to healthy gardenia was without result. Cuttings taken from a gardenia in the early stages of dieback - three from the affected branch but above the lesion and two from a separate branch - remained normal for 22 months. Fourteen normal Belmont cuttings were planted in the same soil in two 10-inch pots in which two gardenias had died from dieback; 20 months later these cuttings were normal and vigorous, indicating no evidence of soil transmission of dieback. Ninety healthy gardenias were grafted or patch-budded with dieback tissue. At least 36 of these formed tissue unions, but only 13 expressed any symptoms of dieback. All grafted or budded plants were held for observation for at least a year, and some were held for more than 2 years. Twenty control gardenias were held on the same bench with the grafts; two of these showed dieback symptoms of the same type as those grafted. Root smothering may develop in gardenias long grown in pots when roots clog the drainage hole. Failure to produce symptoms consistently by grafting, even when union was formed, is not in agreement with the assumption that dieback is caused by a virus. Also the lack of uniformity in the time interval between grafting and appearance of dieback weighs against the hypothesis of virus cause.

In March 1957 a healthy Belmont gardenia in a 6-inch pot was plunged in a pail of water for

---

[1] Pathologist and Agricultural Aid, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

17 days, and all roots were killed.

All symptoms associated with dieback appeared in this plant: bright-yellow veinbanding, brown necrosis along midribs, leaf drop, and black necrosis of twigs. The whole plant was dead 2 months after the roots were killed. Treatment in this test was certainly more drastic than one would expect in commercial greenhouse operations, but it seems likely that dieback is caused by root smothering resulting from overwatering. The chief unexplained discrepancy lies in the fact that when dieback occurs in a commercial greenhouse roots remain white until late stages, whereas in our drastic root-smothering test the roots died first. In pot culture gardenias die when drainage is impaired; they die at different rates just as they do in commercial greenhouses. It seems reasonable that uneven soil texture in benches and beds of greenhouse gardenias, together with heavy watering, may bring on local conditions of suboxidation and then dieback.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

FIRST REPORT OF TREE MORTALITY FROM FOMES ANNOSUS ROOT ROT IN MISSOURI

T. W. Jones and T. W. Bretz[1]

Root rot, caused by Fomes annosus (Fr.) Cke., has been identified as the cause of extensive mortality in a plantation of shortleaf pine, Pinus echinata Mill., in Washington County, Missouri. Although Spaulding collected F. annosus from pine timbers in a clay mine near St. Louis in May 1906[2], this recent find is believed to be the first report of the occurrence of the organism as a parasite in this State.

In the eastern states, where this disease is causing serious losses in some coniferous stands, the 3 to 5 years following thinning appear to be the period during which a stand is most susceptible to root rot invasion. Many acres of natural and artificial pine stands in this State now or soon will require thinning if satisfactory growth is to be maintained, so the disease must be considered a potential threat to Missouri's pine forests.

The infected stand is an old field plantation, 23 acres in extent. The surface soil at this site is a silt loam with a present pH of about 5.1. Shortleaf pine 1-0 stock was planted in April of 1937 at a 6 x 6-foot spacing. In March 1952, 78 percent of the planted trees were living. In a commercial thinning in May-August, 1952, 38 percent of the living trees were removed leaving a residual stand averaging 527 trees per acre. In May 1957, a second thinning reduced the stand per acre to 265 trees. Annual growth since the first thinning has averaged 2.0 feet in height and 0.18 inches d.b.h. per tree.

Extensive mortality in the plantation was first observed in November of 1956, 4 years after thinning. Needle browning and defoliation were associated with rapid decline and death of affected trees. Growth rates appeared normal up to the time of symptom expression. Isolations from the roots of trees in an early stage of decline yielded pure cultures identified as Fomes annosus. This identification was confirmed at the Forest Disease Laboratory, United States Forest Service, Beltsville, Maryland. Through 1957, about 10 percent of the plantation trees had died as a result of root rot infection and additional losses in the future can be expected.

This disease has not been found elsewhere in the State, but surveys are now being made to determine the extent of the root rot problem in Missouri.

CENTRAL STATES FOREST EXPERIMENT STATION, FOREST SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, AND MISSOURI AGRICULTURAL EXPERIMENT STATION

---

[1] Respectively, Pathologist, Central States Forest Experiment Station, Forest Service, United States Department of Agriculture, Columbia, Missouri, and Professor of Forestry, School of Forestry, University of Missouri. Contribution from the Central States Forest Experiment Station, Forest Service, United States Department of Agriculture, and Missouri Agricultural Experiment Station, Journal Series No. 1885. Approved by Director.

[2] Information provided in memorandum dated April 23, 1957, to senior author from Mrs. Frances F. Lombard, Forest Disease Laboratory, United States Forest Service, Beltsville, Maryland.

UNUSUAL RECORDS OF PLANT DISEASES

BIG VEIN OF LETTUCE DISCOVERED IN NEW MEXICO

By Philip J. Leyendecker, Jr.

Lettuce has been grown as a minor commercial crop in the Mesilla Valley of southern New Mexico for a number of years. Because of cotton acreage allotments, the planting of lettuce has increased and spread to other irrigated areas of the State. In 1957, 1400 acres were planted to lettuce, and in 1958 plantings have increased to 2100 acres.

Expanding acreages of lettuce have brought about numerous disease and insect problems. Mosaic and aster yellows virus diseases have appeared, causing serious damage in numerous instances. Fall-planted lettuce has been extremely vulnerable to aster yellows attack.

In 1958, a number of growers in the Mesilla Valley reported unusual-appearing plants in their fall-planted lettuce. Inspection of these fields revealed that the big vein disease was causing the typical upright growth habit accompanied by enlarged, cleared veins and very irregular leaf margins. One field which had grown eight successive lettuce crops was 65 to 70 percent infected. The grower estimated that he lost 100 two-dozen crates of lettuce to the acre.

Heads produced by infected plants were also considerably smaller. In another lettuce field, where there was slightly less infection, the loss amounted to 75 crates per acre. In fields where infection was light, very little, if any, damage occurred. Damage estimates are rather difficult to measure, as they depend upon the season and market conditions. Infected plants usually produce smaller heads, which may be sold if the market demands, or left in the field. Also, infected heads mature less rapidly, making the crop from 7 to 10 days late. In some instances, this may mean the difference between a sizeable profit or none at all.

Big vein disease seemed to be more prevalent in heavy soil and in areas where irrigation water stood for some time.

DEPARTMENT OF AGRICULTURAL SERVICES, NEW MEXICO COLLEGE OF A&MA, STATE COLLEGE, NEW MEXICO

REACTION OF RHUBARB AND BUCKWHEAT TO INOCULATION WITH REED GRASS RUST

By John W. Baxter

Puccinia phragmitis (Schum.) Korn., the common rust of reed grass, Phragmites communis Trin., occurs on this host throughout the eastern and central United States. Proven aecial hosts include several species of Rumex. Aecial material collected in Nebraska on rhubarb has been assigned to P. phragmitis, apparently on the basis of field observations only.

This note reports the results of field and greenhouse inoculations made during April and May, 1958, with overwintered telial material of Puccinia phragmitis collected near Bayard, Nebraska. This material was used to inoculate rhubarb, Rheum raponticum L., curly dock, Rumex crispus L., and buckwheat, Fagopyrum esculentum Moench. Field inoculations of rhubarb and curly dock were made by spraying young leaves with water and enclosing each leaf in a plastic bag containing telial material which had been soaked in water for 8 hours. Abundant infection was obtained on both hosts. Greenhouse inoculations of buckwheat were made by suspending soaked telia-bearing leaves of Phragmites above young buckwheat plants in a moist chamber. Due to unfavorable greenhouse temperatures most of the plants used in these inoculations died 2 or 3 days after being removed from the moist chamber. Infection was obtained on a single leaf of one surviving plant. This infection was first evident as a yellowish spot near the margin of the leaf. Aecia appeared approximately 20 days after inoculation. There was no evidence of the formation of spermagonia.

Specimens of aecial material resulting from these inoculations have been deposited in the Arthur Herbarium, Purdue University.

UNIVERSITY OF WISCONSIN--MILWAUKEE, MILWAUKEE, WISCONSIN

BOOK REVIEW

"DISEASES OF TOBACCO", by George Blanchard Lucas. viii + 498 pages. 1958. The Scarecrow Press, Inc., New York 10, N. Y. Price $10.00.

If a 500-page book on the diseases of a specialty crop like tobacco needs justification for its existence, then Lucas' first sentence, "Tobacco means money" can speak for itself. But, apart from the economic importance of tobacco, there are many other things to recommend this book. Contrary to the usual situation of a very limited reader range for a one-crop book, Diseases of Tobacco should circulate widely because it is admirably suited for use either as a text, as a reference source, or as a handbook in the field -- an unusual combination in any scientific work. Students should be delighted with the easy-to-read style that combines scientific accuracy with clarity, brevity and interest; the research worker will discover a wealth of pertinent, timely facts about tobacco diseases to assist him in his own investigations; and tobacco extension workers and farmers will appreciate the usefulness of a book that in one single volume answers their two most pressing problems: how to recognize a disease, and how to control it.

The author has divided the book into eight parts. The first part is general and includes an introductory chapter and three more chapters devoted to plant beds in relation to control, to rotation, and to breeding for resistance. For the other divisions, one part each is given to the following types of diseases: nematode, fungus, bacterial, virus, diseases caused by flowering plants, malnutrition, and miscellaneous. Each disease is alloted a chapter which begins with a brief introduction, followed usually by symptoms and signs of the disease, etiology, host-parasite relations, the influence of environment, and control. For some diseases sections on history, taxonomy of the causal organism, means of spread of the pathogen, and the like are also included. References are listed at the end of each chapter and there is what appears to be a complete index at the end of the book.

It is regrettable that a work of such obvious intrinsic merit should be marred by inferior production, but such is undoubtedly the case. Offset printing admittedly has its drawbacks, but that is not sufficient excuse for the numerous typographical errors that abound in the text. Lesser mistakes include bad spacing, smudged or faint letters, the omission or addition of letters to words, and, in one case, the repeating of an entire sentence (page 77, middle of page). But even more serious is the occasional misspelling of scientific names and terms, which is always unwelcome, to author and reader alike. Other errors noted were the omission from the bibliography list in Chapter 30 of reference number 12 (which was cited), and in seven other chapters references were found to be out of alphabetical order; the latter oversight, however, is not too serious.

Despite the mechanical shortcomings just mentioned, the reviewer unhesitatingly and wholeheartedly recommends this book. In regard to subject matter, the many years of tobacco disease research in the author's background certainly qualify him as an expert in the field, while his equally important facility for extracting all of the important facts from the myriad of research data available and weaving them into an interesting and unified whole equip him to be an author as well.

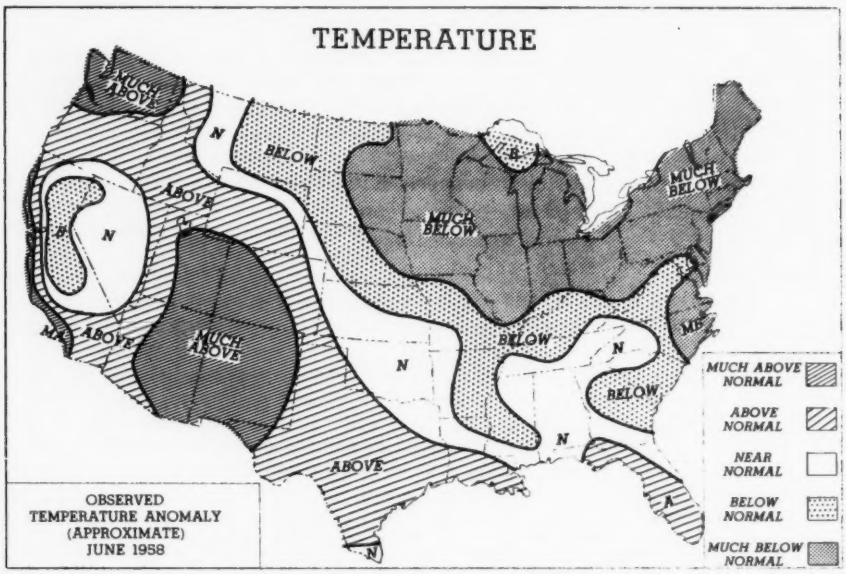
The material included in the book represents an almost unbelievable amount of research work on tobacco. Lucas has made substantial contributions from his own work on Granville wilt, on mosaic, and on black shank. For other diseases he has selected the most valuable contributions of the outstanding men who have worked on the various diseases.

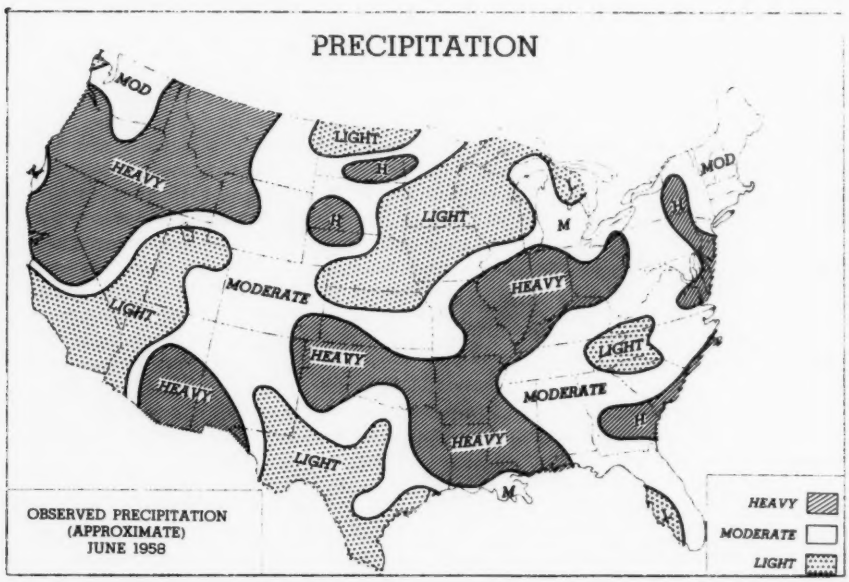
Most of the photographs are excellent. The majority of the 156 figures are photographs of symptoms, but there are also quite a number of photo- and electron-micrographs depicting growth of the pathogen within host tissue, as well as drawings illustrating taxonomic relationships, structural formulas, and so forth. There is a drawing of the nematode responsible for each nematode disease and for most of the diseases caused by fungi there is either a drawing or a photo-micrograph of at least the fruiting body of the fungus involved.

It is sincerely to be hoped that the comparatively high price of this book does not prohibit its purchase, since even a cursory evaluation of the contents will soon prove to the prospective buyer that Diseases of Tobacco deserves a permanent place on the shelves of all plant pathologists and other persons in any way connected with the growing of tobacco. -- HILDE McGRATH

ANNOUNCEMENT
NEW LOCATION OF CHICAGO MARKET PATHOLOGY LABORATORY

The Market Pathology Laboratory, Agricultural Marketing Service, United States Department of Agriculture, which has been located at Chicago University for nearly 40 years, will move to 536 South Clark Street, Chicago 5, Illinois, on or about July 21, 1958. Dr. G. B. Ramsey, Principal Pathologist, is in charge of this laboratory.





The terms used in the accompanying maps, which define the ranges of temperature and precipitation, are numerical class limits. These are based on a statistical analysis of past records through which is determined the normal frequency of occurrence of temperatures and precipitation at various times of the year for different locations. For temperature the classes above, below, and near normal are so defined that they each normally occur one-fourth of the time; much above and much below normal, one-eighth of the time. Precipitation is depicted in terms of light, moderate, and heavy, each class normally occurring one-third of the time and thereby having equal probability of occurrence. These maps graphically represent only the general trends and give the country's weather picture at a glance. For quantitative studies, where monthly mean temperatures and actual precipitation records are needed for a given time and place, other publications of the Weather Bureau should be consulted. P. R. M.